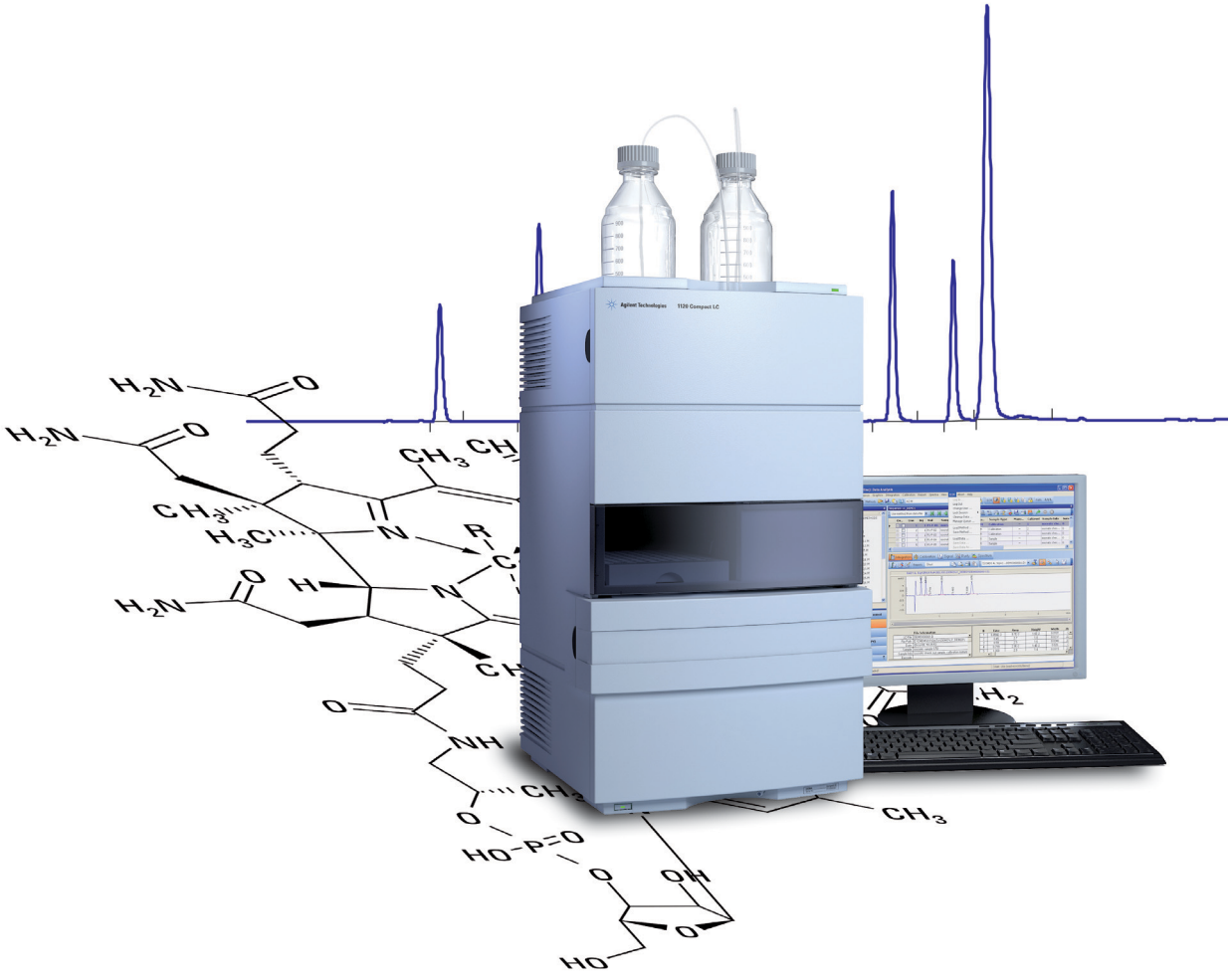
Agilent 1220 Infinity LC

Solutions for Easy-to-Use and Affordable UHPLC

Application Compendium



Agilent Technologies

Table of Contents

Introduction

This compendium is a collection of technical information and applications performed on the Agilent 1220 Infinity LC and its predecessor the Agilent 1120 Compact LC.

Based on well-established Agilent LC technologies, the 1120 Compact LC featured simplified functions and easy-to-use software to facilitate conventional HPLC up to 400 bar. The 1120 Compact LC was mainly deployed in small to medium sized quality control or research laboratories.

A customer survey conducted by Agilent revealed the requirements for more instrument flexibility and a wider power range. In response Agilent introduced the 1220 Infinity LC, leveraging the latest technology by sharing components with the Agilent 1260 Infinity LC while maintaining excellent value and compatibility.

Key Features

- Pressure range up to 600 bar – for UHPLC in combination with Agilent Poroshell columns
- Integrated diode array detector with spectra analysis – for impurity characterization
- Hardware upgrade paths such as isocratic to gradient for higher method flexibility, manual to automated injection for higher sample throughput, and a click-in column oven for better chromatographic reproducibility
- Seamless addition of any Agilent LC detector or analytical-scale fraction collector, including integrated software control
- Scalable software choices from basic workstation packages to distributed client/server solutions, including options for regulatory compliance
- A special mounting plate absorbs shocks and vibration during transport or operation for onsite analysis in remote areas

Performance of the Agilent 1220 Infinity LC

The first part of the Agilent 1220 Infinity LC Application Compendium demonstrates the typical performance of the 1220 Infinity LC. This includes high precision of retention time and area, precise gradient formation, and excellent signal-to-noise ratio.

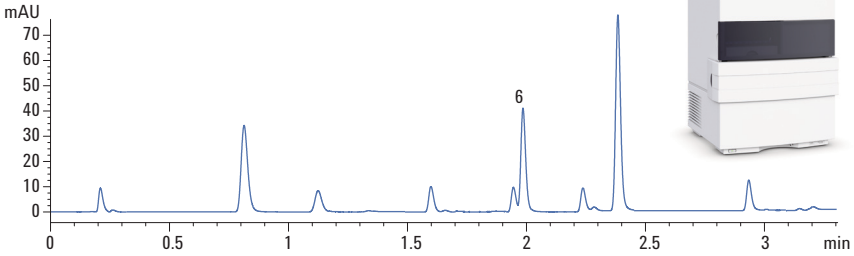
Additional technical details describe the integration of the 1220 Infinity LC with third party software related to instrument control.

Furthermore, analysis in combination with fraction collection and analysis of PAHs with fluorescence detection show the flexibility of the 1220 Infinity LC.

Performance characteristics of the Agilent 1220 Infinity Gradient LC System

An integrated LC system for conventional LC and UHPLC

Technical Overview



Introduction

The Agilent 1220 Infinity Gradient LC is a liquid chromatography (LC) system for routine standard analysis. Due to its extraordinary pressure range up to 600 bar, the system can also perform UHPLC applications. It is an integrated LC system consisting of a binary low pressure mixing ('dual gradient') pump, autosampler, column compartment and variable wavelength detector (VWD). The dual gradient pump has a flow range from 0.2 up to10 mL/min (5 mL at 600 bar, 10 mL at 200 bar), low pressure mixing and an integrated degasser. The VWD detector features 80 Hz data acquisition rate and a wavelength range from 190 nm up to 600 nm. The autosampler can operate up to 600 bar, with an injection volume range from 0.1 to 100 µL and a capacity of 100 2 mL vials. The column oven holds one 25 cm column and the maximum temperature is 60 °C.

In this Technical Overview the following parameters were tested on the Agilent 1220 Infinity Gradient LC:

The pump was tested for:

- Precision of retention time
- Performance of step gradients

The autosampler was tested for

- Precision of areas
- Carryover
- Injection volume linearity

The variable wavelength detector was tested for:

- Noise and drift
- Linearity

Method transfer from conventional to UHPLC is another important application which will be demonstrated.

Experimental

The Agilent 1220 Infinity LC Gradient System (G4290B) was equipped with dual gradient pump, autosampler, column compartment and the variable wavelength detector.

Pump performance–Precision of retention times

Optimum retention time precision depends mainly on:

- Pump performance, the most important issue
- Equilibration status of the columns
- Equilibration status of the complete system
- Degassing of the solvent
- Temperature stability of the column compartment

The most important parameter is the pump performance itself, however, other parameters can influence precision of retention times. For example, if a solvent was changed the column needs at least ten column volumes for proper equilibration. If gradients are applied, at least five column volumes are needed to equilibrate the column to the start conditions. Furthermore, if the column compartment temperature was changed, for example from 30 °C to 60 °C, it requires approximately 20 minutes until the column is equilibrated to the new temperature. Proper degassing influences the precision positively.

In this Technical Overview, retention time precision was tested with different gradient and isocratic conditions using 4.6 and 3 mm internal diameter (id) columns. The relative standard deviation (RSD) for retention times is typically < 0.2% RSD for gradient analysis. In Figure 1, an example of an isocratic application is shown. Here the flow precision is typically < 0.07% RSD for retention times.

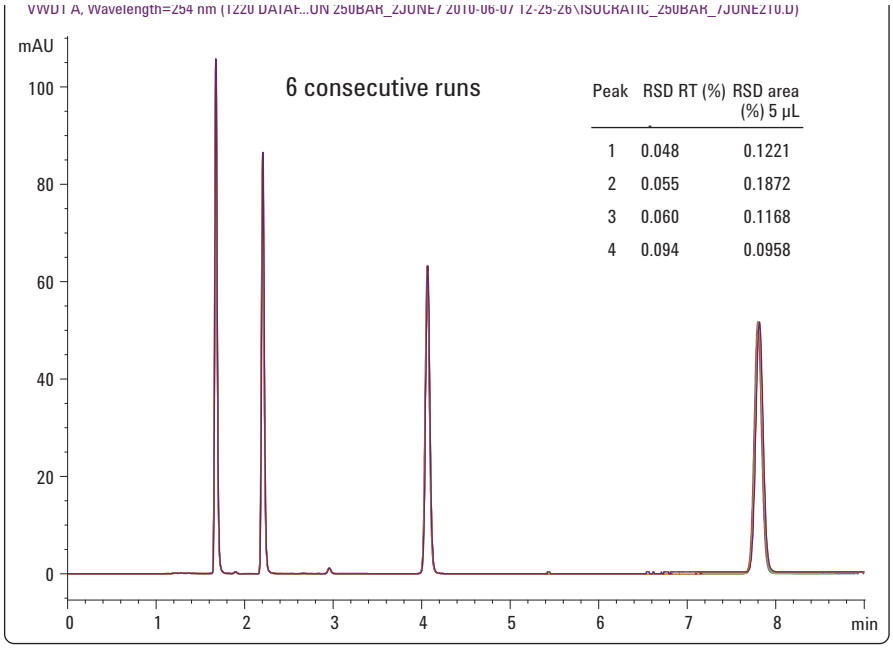


Figure 1
Retention time and area precision for an isocratic run run with dynamic mixing.

Sample	Isocratic test sample (p/n 01080-68707)
Column	Agilent ZORBAX Eclipse Plus C18 4.6 × 150 mm, 1.8 µm (PN 959994-902), 245 bar
Mobile phase	A = Water B = Acetonitrile
Isocratic	30/70 A/B
Flow rate	1.2 mL/min
Stop time	9 min
Injection volume	5 µL , draw speed 200 µL/min
Column temperature	40 °C
VWD	254 nm
Flow cell	10 mm
Peak width:	PW 0.05 min (10 Hz)

In Figure 2 an example for a gradient run is shown. The retention time precision is < 0.045%RSD, except for the first peak

In Figure 3 an example for a fast gradient run at 563 bar is shown. In this application Poroshell columns were used. The retention time precision is < 0.15%RSD.

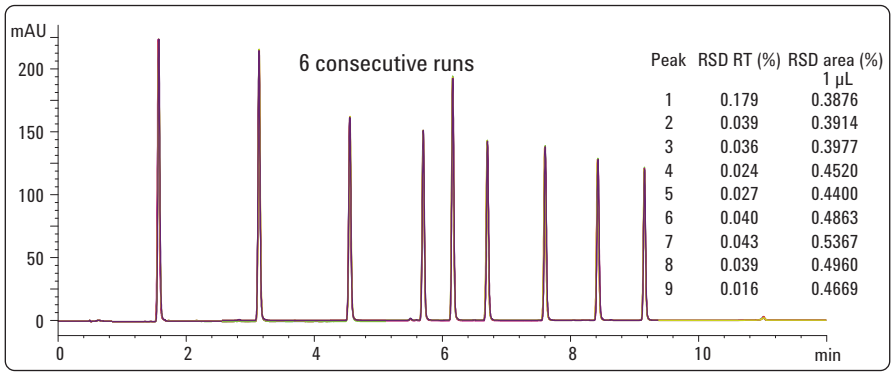


Figure 2
Precisions for a gradient run at 445 bar with a RT precision < 0.045% RSD except for the first peak.

Sample	RRLC checkout sample (p/n 5188-6529)
Column	Agilent ZORBAX Eclipse Plus C18 3 × 100 mm, 1.8 µm
Mobile phase	A = Water
	B = Acetonitrile
Gradient	20% B to 95% B in 10 min
Flow rate	1 mL/min
Stop time	12 min
Post time	5 min
Injection volume	1 µL
Column temperature	40 °C
VWD	245 nm
Flow cell	10 mm
Peak width	> 0.05 min (10 Hz)

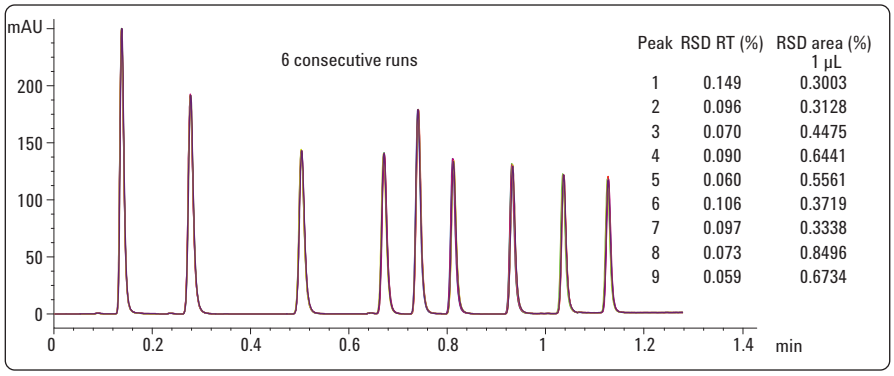


Figure 3
Retention time and area precision at 563 bar for a fast gradient application. RSD RT is < 0.15.

Sample	RRLC checkout sample (p/n 5188-6529)
Column	Agilent Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm
Mobile phase	A = Water
	B = Acetonitrile
Gradient	30% B to 95% B in 1 min
Flow rate	3.5 mL/min
Stop time	1.5 min
Post time	1 min
Injection volume	1 µL
Column temperature	40 °C
VWD	245 nm
Flow cell	10 mm
Peak width	PW > 0.0125 min (40 Hz)

Performance of step gradient

Tracer experiments are also frequently used to verify the solvent mixing ripple at different gradient mixtures, to evaluate pump performance. The delay volume, the accuracy and precision of gradients are also evaluated using step gradients. Figure 4 shows a step gradient from 0 to 100% in 10% steps. Caffeine was selected as the tracer compound. Acetone is not ideal for testing step gradient performance because acetone is too easily removed in the degasser at low flow rates. For testing the step gradient performance of the Agilent 1120 Infinity LC, we recommend using non-volatile compounds.

The performance results are :

- Ripple on 10% step = 0.03%
- Ripple on 50% step = 0.08%
- Ripple on 90% step = 0.08%
- Precision of step height for 50% step = <0.1%RSD for 3 runs
- Delay volume: 860 µL

Injector performance–Area precision

Precise injection is mandatory for good quantitative results in liquid chromatography. The Agilent 1220 injector can inject precisely over an injection range from 0.5 up to 100 µL. Examples are given in Figures 1 to 3 . In Figure 1 the Area precision is < 0.19% RSD for 5 µL injection volume. Figure 2 shows an example of a conventional gradient. The area precision for 1 µL is < 0.54% RSD. The area precision for 1 µL is <0.85% RSD for a fast gradient run as shown in Figure 3.

The injector settings are very important for optimum precision of areas. For example, if highest precision is needed the draw speed of the injector should be set to lower values, especially if large volume or viscous sample are injected.

Carryover

Carryover was tested using the built in Agilent 1220 autosampler. The injection draw speed was set to 20 µL/min and an exterior needle wash was used. The carryover (Figure 5) was found to be < 0.031% for the conditions used. After a 500 ng sample injection, unadulterated solvent was injected.

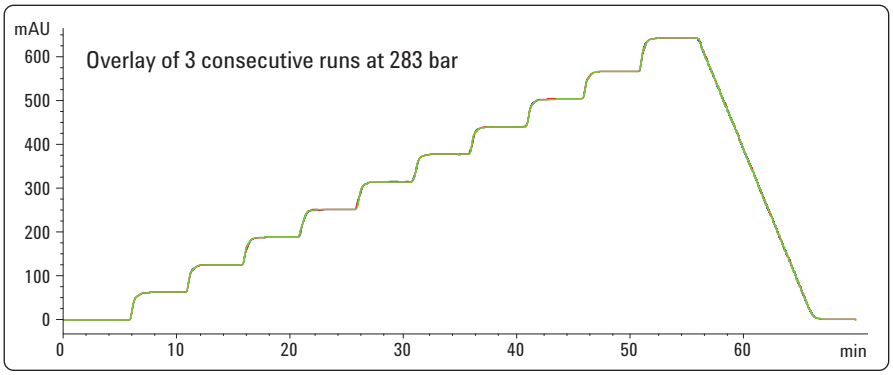


Figure 4
Overlay of 3 step gradients.

Column	Restriction capillary with 283 bar backpressure
Mobile phase	A = Water +20% Isopropanol
	B = Water + 20% Isopropanol +10 mg/L Caffeine
Step gradient	from 0% to 100% B in 10% steps
Flow rate	1 mL/min
Stop time	70 min
Post time	5 min
Column temperature	36 °C
VWD	273 nm
Flow cell	10 mm
Peak width	> 0.025 min (20 Hz)

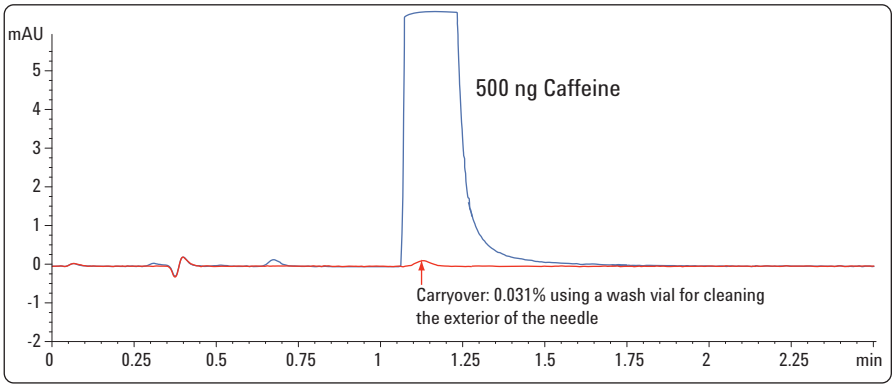


Figure 5
Carryover of 500 ng injection of Caffeine is 0.031%.

Sample	Caffeine 500 ng/µL
Column	Agilent Poroshell 120 EC C-18 3 × 50 mm, 2.7 µm
Mobile phase	A = Water
	B = Acetonitrile
Isocratic	10% B
Flow rate	0.8 mL/min
Stop time	2.5 min
Injection volume	1 µL , draw speed 20 µL/min, wash vial in position 41
Column temperature	30 °C
VWD	273 nm
Flow cell	10 mm
Peak width	PW > 0.025 min (20 Hz)

Injection volume linearity

Injection volume linearity was tested using Primidone standards. All injection volumes contained 781.26 ng of Primidone. The injection volume varied but the injected amount was the same, (Figure 6). The peak heights and areas are expected to be the same for all injection volumes. The experiments show that all areas were within 0.82% RSD over the complete injection volume range of 0.8 to 100 µL. Each injection volume was injected 3 times and the resulting 24 runs were evaluated for area precision.

Prepare an accurate dilution series to obtain good linearity. One way is to dilute large volumes, for example starting with one liter. If only small volume should be diluted special care has to be taken: The pipettes should be calibrated, and the same pipette should be used for the complete dilution series. Otherwise there is a big risk that a dilution error is measured rather than linearity.

Detector performance

Evaluation of baseline noise according to guidelines of the American Society for Testing and Materials (ASTM) and drift of the 10 mm and 60 mm path length cell.

ASTM noise and drift was evaluated using a restriction capillary instead of a column and water as the mobile phase. The variable wavelength detector was set to a four seconds response time. The resulting ASTM noise of the 10 mm path length cell was found to be ±2.2 µAU and the drift was1.2 mAU/h (Figure 7).

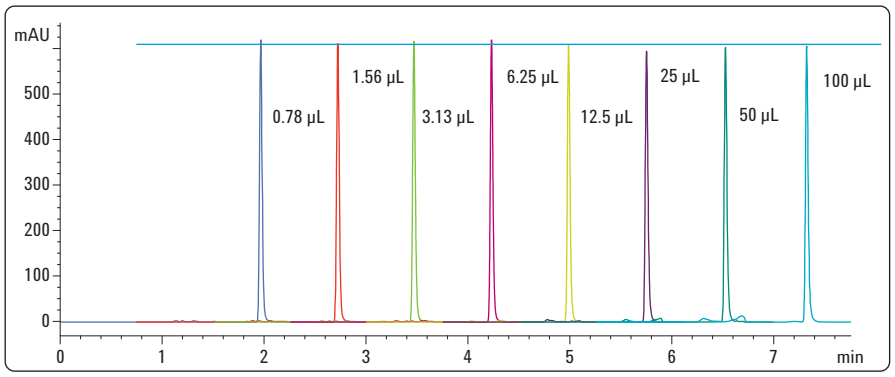


Figure 6
Injection volume linearity from 0.8 to 100 µL using Primidone as test compound.

Sample	Primidone 25 mg/25 mL, 7 times 1:2 diluted
Column	Agilent ZORBAX Eclipse Plus C18 150 × 4.6 mm,1.8 µm
Mobile phase	A = Water B = Acetonitrile
Isocratic	30% B
Flow rate	0.8 mL/min
Stop time	2.5 min
Injection volume	0.78 to 100 µL , draw speed 50 µL/min
Column temperature	40 °C
VWD	220 nm
Flow cell	10 mm
Peak width	PW > 0.025 min (20 Hz)

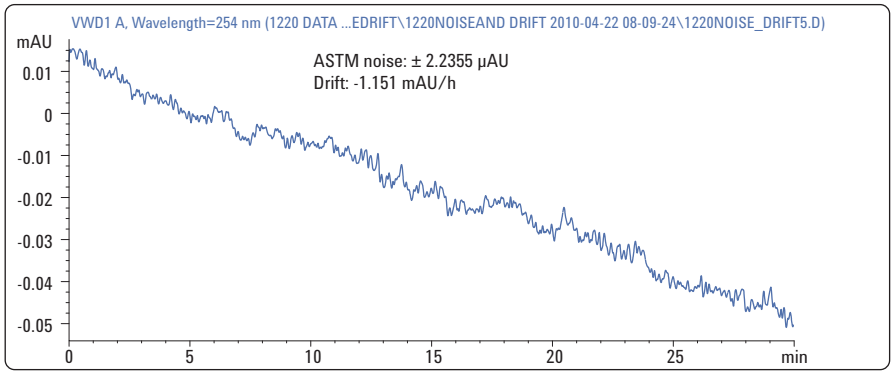


Figure 7
VWD noise and drift measurement.

Column	Restriction capillary with 42 bar backpressure
Mobile phase	A = Water isocratic
Flow rate	1 mL/min
Stop time	30 min
Column temperature	36 °C
VWD	254 nm
Flow cell	10 mm
Peak width	> 0.2 min (4 s response) (2.5 Hz)

Linearity for different caffeine concentrations

Linearity was tested using caffeine standards from 1.5 ng to 750 ng of injected amount. For this concentration range, very good linearity was obtained. The coefficient of correlation was 0.99996. The response factors were all within the 5% error range from 1.5 up to 750 ng (Figure 8).

Method transfer from conventional to UHPLC

In this experiment, columns of different pore size and length were employed to shorten analysis and optimize resolution (Figure 9).

It has been shown that the Agilent 1220 Infinity LC system is an instrument that can be used for conventional chromatography (Figure 9, blue trace) as well as UHPLC (other traces). It is possible to progress step by step to UHPLC and optimize for resolution (Figure 9, green trace) or for speed (pink trace).

Chromatographic conditions

Sample from Sigma Aldrich:

- Reversed Phase Test Mix (Order No.: 47641-U)
- 1 × 1 mL (uracil, phenol, n,n-diethyl-m-toluamide, toluene)
- HPLC Gradient System Diagnostic Mix (Order No.: 48271)
- 6 × 1 mL (phenol, methyl parabens, ethyl parabens, propyl parabens, butyl parabens, hepthyl parabens, uracil)

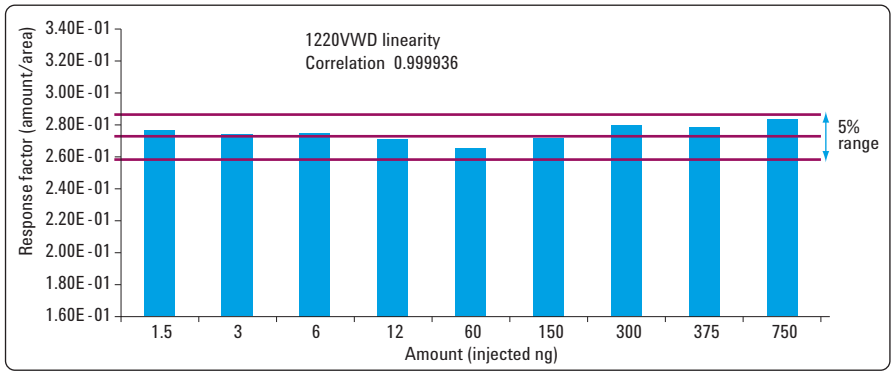


Figure 8
VWD linearity using Caffeine as sample compound.

Sample	Caffeine standards
Column	Agilent Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm
Mobile phase	A = Water
	B = Acetonitrile
Isocratic	10% B
Flow rate	0.8 mL/min
Stop time	1.5 min
Post time	1 min
Injection volume	3 µL
Column temperature	30 °C
VWD	273 nm
Flow cell	10 mm
Peak width	PW 0.025 min (20 Hz)

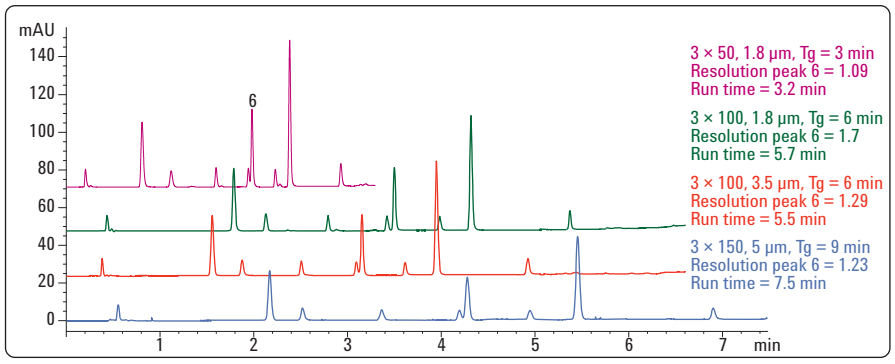


Figure 9
Optimization of resolution and speed by changing step by step from conventional to UHPLC.

Sample preparation
Dilute each sample to 5 mL with water/acetonitrile 1:1. Mix the two diluted samples 1:1

Column	Agilent ZORBAX Eclipse Plus C18, 3 x 50, 1.8 µm, 3 x 100, 1.8 µm, 3 x 100, 3.5 µm 3 x 150, 5 µm
Mobile phase	A = Water
	B = Acetonitrile
Gradient:	0 min 20 % B
	3 min, 6 min, 6 min, 9 min 100% B
Flow rate	1.2 mL/min
Stop time	3.2 min, 5.7 min, 5.5 min, 7.5 min
Injection volume	3 µL
Column temperature	40 °C
VWD	254 nm
Flow cell	10 mm
Peak width	> 0.005 min (80 Hz)

It is also possible to obtain better resolution in less time by further increasing the flow rate, (Figure 10).

Chromatographic conditions

Sample from Sigma Aldrich:

Reversed Phase Test Mix (Order No.: 47641-U)

1 × 1 mL (uracil, phenol, n,n-diethyl-m-toluamide, toluene)

HPLC Gradient System Diagnostic Mix (Order No.: 48271)

6 × 1 mL (phenol, methyl parabens, ethyl parabens, propyl parabens, butyl parabens, hepthyl parabens, uracil)

Sample preparation:

Dilute each sample to 5 mL with water/acetonitrile 1:1

Mix the two diluted samples 1:1

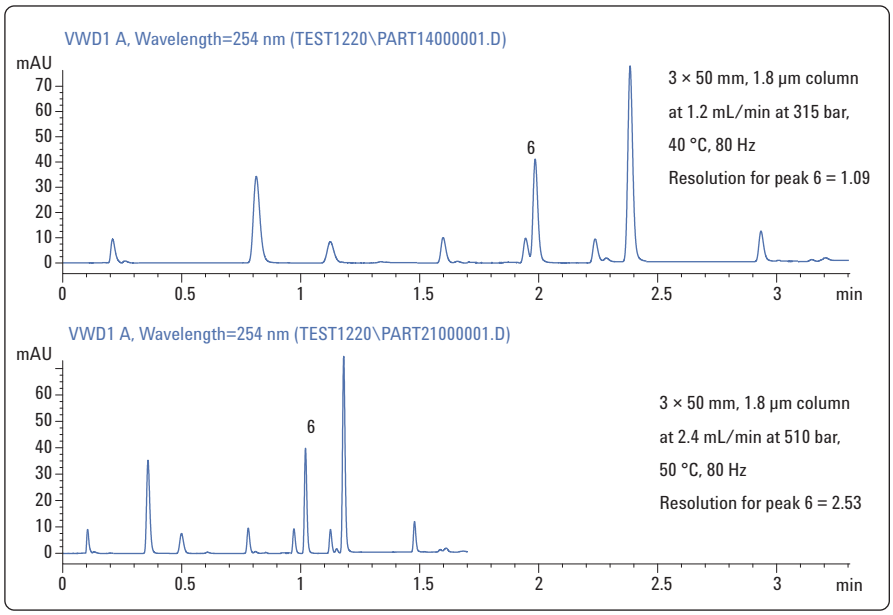


Figure 10
Improving speed and resolution by increasing the flow rate.

Column	Agilent ZORBAX Eclipse Plus C18 3 × 50 mm, 1.8 µm
Mobile phase	A = Water
	B = Acetonitrile
Gradient	0 min 20 % B
	3 min 100 % B
	3.3 min 100 % B
Flow rate	1.2 mL/min
Stop time	3.3 min
Post time	3 min
Injection volume	3 µL
Column temperature	40 °C
VWD	254 nm
Flow cell	10 mm
Peak width	> 0.003 min (80 Hz)

Column	Agilent ZORBAX Eclipse Plus C18 3 × 50 mm, 1.8 µm
Mobile phase	A = Water
	B = Acetonitrile
Gradient	0 min 20 % B
	1.5 min 100 % B
	1.7 min 100 % B
Flow rate	2.4 mL/min Pressure
Stop time	1.7 min
Post time	2 min
Injection volume	3 µL
Column temperature	50 °C
DAD	254 nm / 4 (360 nm / 100)
Flow cell	10 mm
Peak width	> 0.003 min (80 Hz)

Conclusion

The performance of the Agilent 1220 Gradient LC system fulfills all needs of modern analytical liquid chromatography. It is especially well suited for 3 mm and 4.6 mm id columns and can be used for conventional and for rapid resolution (RR) or ultra fast LC on columns packed with 1.8 µm particles.

Precision of retention times is typically </= 0.2% RSD. The precision for areas is typically < 0.25% for injection volumes >/= 5 µL. Carryover is typically < 0.05% with external needle cleaning. The VWD combines lowest noise (ASTM noise for the 10 mm path length cell was found to be ± 3.5 µAU) with a linear range up to 2 mAU.

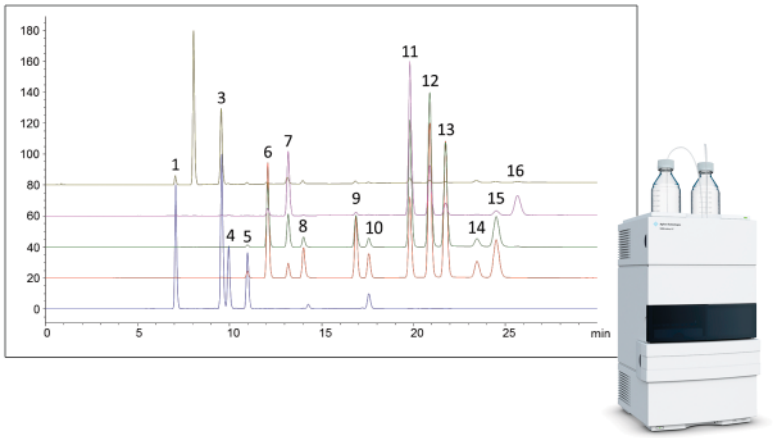
Proof of Performance

Enhancing the capabilities of the Agilent 1220 Infinity LC System with the Agilent 1260 Infinity Fluorescence Detector

Technical Overview

Author

Udo Huber
Agilent Technologies, Inc.
Waldbronn, German



Abstract

The Agilent 1220 Infinity LC is an integrated, robust and easy-to-use system for HPLC and UHPLC. Capable of handling back pressures up to 600 bar at flow rates up to 5 mL/min, the 1220 Infinity LC is ideal for UHPLC applications that deploy sub-2-µm or superficially porous columns with an id of 3 – 4.6 mm. In addition, instrument-to-instrument method transfer has been implemented by design in the 1220 Infinity LC, enabling the system to run legacy methods from any Agilent 1100 Series, Agilent 1200 Series, or Agilent 1200 Infinity Series instrument. In this Technical Overview, we demonstrate how the integrated 1220 Infinity LC can be enhanced with an external Agilent 1260 Infinity Fluorescence Detector and how a method for the analysis of polynuclear aromatic hydrocarbons can be transferred easily from an Agilent 1260 Infinity LC system.

Introduction

Polynuclear aromatic hydrocarbons (PAHs) are hydrocarbons with multiple ring structures. This class of compounds is suspected to be mutagenic and/or contain toxic compounds. As a result, many countries have introduced maximum permission levels, which has led to the need for suitable monitoring methods. Such HPLC and UHPLC methods were described in a previous Agilent Application Note[1]. Detection using a fluorescence detector (FLD) with its high selectivity and sensitivity has proven to be ideal for the quantitative analysis of PAHs.

The Agilent 1220 Infinity LC system is ideally suited for this type of analyses but does not have an integrated FLD. Using the Controller Area Network (CAN) it is easy to connect any other Agilent module to the 1220 Infinity LC system. In this Technical Overview, the CAN connection was used to connect an FLD to the 1220 Infinity LC system for the analysis of PAHs. To demonstrate the instrument-to-instrument method transfer by design, the retention times of the PAH compounds measured on the 1220 LC system and on a 1260 Infinity LC system from a previous Application Note[1] were compared.

Experimental

Instruments and software

An Agilent 1220 Infinity LC Gradient System (G4290B), including a gradient pump (max. pressure 600 bar) with integrated degasser, autosampler, column oven, and variable wavelength detector with standard flow cell (10 mm path length) was used.

The Agilent 1200 Infinity Series Fluorescence Detector (G1321B) was equipped with the standard flow cell (8 µL) and connected via CAN to the 1220 Infinity LC.

The system was controlled using the Agilent OpenLAB CDS ChemStation Edition Rev. C.01.03.

Samples and solvents

SS EPA 610 PAH Mix in Methanol/ Methylene Chloride (1:1), (Supelco Analytical), containing the following PAHs: Naphtalene, Acenaphtylene, Acenaphtene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(b)fluoranthene, Benzo(k) fluoranthene, Benzo(a)pyrene, Dibenzo(a,h)anthracene, Benzo(g,h,i) perylene and Indeno(1,2,3-cd)pyrene

Acetonitrile was LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak).

Chromatographic conditions

Parameter	Setting
Column:	Agilent ZORBAX Eclipse PAH 4.6 x 150 mm, 5 µm
Mobile phase:	A = Water, B = Acetonitrile
Gradient:	at 0 min 40% B at 20 min 95% B at 30 min 95% B
Stop time:	30 min
Post time:	10 min
Flow rate:	1.5 mL/min
Injection volume:	3 µL
Column temp.:	25 °C
VWD:	230 nm
FLD:	Emission: 260 nm Excitation: 350, 420, 440, and 500 nm (A, B, C, D)

Results and discussion

The PAH sample was analyzed on a dedicated PAH column applying a gradient using water and acetonitrile as mobile phase. Since the PAHs have their emission maximums at different wavelengths, the multi-signal acquisition feature of the Agilent 1260 Infinity FLD was applied. In addition, the UV signal of the integrated variable wavelength detector (VWD) of the Agilent 1220 Infinity LC system was used for the analysis of acenaphthylene.

Figure 1 shows the multisignal chromatogram for the analysis of PAHs using fluorescence detection.

A major advantage of the 1220 Infinity LC system is the "instrument-to-instrument method transfer by design", which means that methods from any Agilent 1100 Series, Agilent 1200 Series, or Agilent 1200 Infinity LC system can be transferred without modifications and lead to the same results, for example, the same retention time. In this Technical Overview, the transfer was even done from an Agilent 1260 Infinity Series Binary Pump, which is a high-pressure mixing pump, to the 1220 Infinity System, with a built-in low-pressure mixing pump. But even when the transfer is done from a pump using a different operation and mixing principle[2], the retention time differences are 2.2 % or less as shown in Table 1. While the experiments on the 1260 Infinity LC and the 1220 Infinity LC system were done on the identical column, several weeks passed and many injections were meanwhile done on that column. Therefore, this value lies well within the limits that would be expected from column aging over this time.

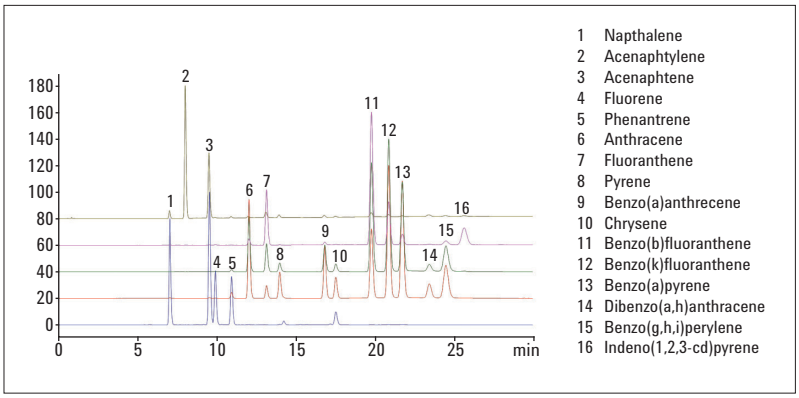


Figure 1
Multi-signal chromatogram for the analysis of PAHs using fluorescence detection (acenaphthylene was determined using UV detection).

Compound	Signal	RT (1260 Infinity)	RT (1220 Infinity)	Δ (%)
Naphthalene	FLD A	7.24	7.08	2.2
Acenaphthylene	VWD	8.24	8.06	2.2
Acenaphthene	FLD A	9.72	9.57	1.5
Fluorene	FLD A	10.1	9.95	1.5
Phenanthrene	FLD A	11.11	10.97	1.3
Anthracene	FLD B	12.22	12.07	1.2
Fluoranthene	FLD C	13.34	13.17	1.3
Pyrene	FLD B	14.16	14.00	1.1
Benzo(a)anthracene	FLD B	16.95	16.86	0.5
Chrysene	FLD B	17.63	17.56	0.4
Benzo(b)fluoranthene	FLD C	19.82	19.82	0.0
Benzo(k)fluoranthene	FLD B	20.86	20.90	0.2
Benzo(a)pyrene	FLD B	21.68	21.76	0.4
Dibenzo(a,h)anthracene	FLD B	23.37	23.46	0.4
Benzo(ghi)perylene	FLD B	24.47	24.50	0.1
Indeno(1,2,3-cd)pyrene	FLD D	25.75	25.66	0.3

Table 1
Retention time differences between the Agilent 1260 Infinity Binary LC System and the Agilent 1220 Infinity LC System.

Conclusion

In this Technical Overview, we have shown the enhancement of an Agilent 1220 Infinity LC system by simply adding an Agilent 1260 Infinity FLD through CAN bus connection. Using the analysis of PAHs as an example, we could also demonstrate the simple method transfer between systems leading to identical results.

References

1
"Agilent Application Solution: Analysis of PAHs in soil according to EPA 8310 method with UV and fluorescence detection" Agilent Technologies Application Note, **2011**, publication number 5990-8414EN

2
"Scope of low and high pressure mixing Comparing the Agilent 1260 Infinity Quaternary and Binary Pumps" Agilent Technologies Technical Overview, **2011**, publication number 5990-7143EN

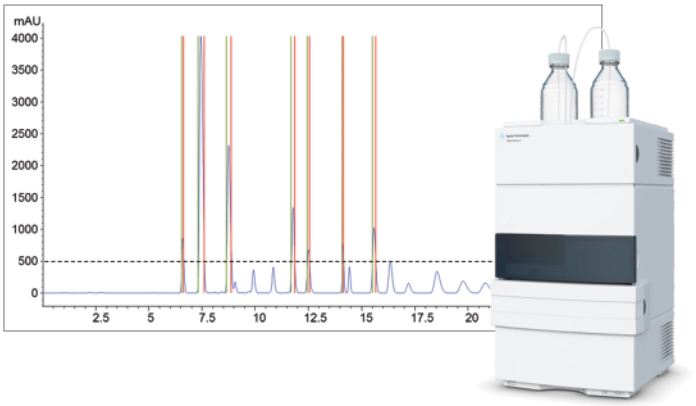
Proof of Performance

Time- and peak-based fraction collection with the Agilent 1220 Infinity LC System

Technical Overview

Author

Udo Huber
Agilent Technologies, Inc.
Waldbronn, Germany



Abstract

Analytical-scale purification of compounds can be performed easily using standard HPLC or UHPLC equipment that is capable of delivering flow rates up to 10 mL/min. In this flow rate range, columns with ids from 4 up to 10 mm can be operated close to their *vanDeemter* optimum, facilitating the purification of crude compounds at amounts between 1 and 10 mg. In this Technical Overview, we demonstrate the combination of the Agilent 1220 Infinity LC System with the Agilent 1260 Infinity Analytical-scale Fraction Collector for time- and peak-based fraction collection using a generic compound mixture. Triggering of fractions is done based on retention time windows as well as based on the signal of the built-in variable wavelength detector (VWD) of the 1220 Infinity LC System. The collected fractions are re-analyzed to demonstrate the purity of the isolated compounds.

Introduction

Preparative LC is not defined by column diameters or flow rates but by what happens to the compounds after they are separated on the column. While in analytical LC, the compounds are passed to waste after the last detector in the system, in preparative LC, they are transferred to a fraction collector. Based on selectable triggering criteria such as retention time windows or detector signals, the fraction collector transfers the desired parts of the chromatogram to dedicated fraction containers, for example, tubes, vials, or well plates. To ensure that only the desired part of the chromatogram such as a specific peak is collected with high purity and recovery, two prerequisites have to be fulfilled by the system:

- The first requirement is an accurate determination of the delay time of the system. When a compound is detected by the detector, the fraction collector has to wait until the compound has travelled to the fraction collector diverter valve. This delay time has to be determined beforehand. Manual procedures usually involve some sort of a dye and a stop watch — but they are usually very tedious, unreliable, and inaccurate. With the patented Agilent Delay Sensor[1] and a protocol executed within the Agilent LabAdvisor software, the delay calibration is a simple, fast, and accurate task.
- The second requirement is minimized system dispersion between the detector and the fraction collector. Unnecessarily long or wide capillaries lead to broadening of the peak travelling from the detector to the fraction collector due to dispersion effects. Since the time the diverter valve of the fraction collector remains in the collect position is determined by the width of the peak in the detector, excessive peak broadening can lead to decreased recovery and purity. The Agilent 1260 Infinity Analytical-scale Fraction Collector was especially designed to minimize system dispersion by using optimized connection capillaries and by positioning the diverter valve as close as possible to the fraction collection needle.

While both requirements have to be fulfilled for any preparative LC system, they are especially important when working at low flow rates such as used in analytical-scale preparative LC.

Experimental

Instruments and software

An Agilent 1220 Infinity LC Gradient System (G4290B), including a gradient pump (max. pressure 600 bar) with integrated degasser, autosampler, column oven, and variable wavelength detector with standard flow cell (10 mm path length) was used.

Fractions were collected using an Agilent 1260 Infinity Analytical-scale Fraction Collector (G1364C), equipped with a 4-well plate tray and four vial plates for 54 × 2 mL vials.

The system was controlled using the Agilent OpenLAB CDS ChemStation Edition Rev. C.01.03.

The delay calibration was performed using the Agilent Lab Advisor Software Rev. B.02.01.

Samples and solvents

SS EPA 610 PAH Mix in Methanol/ Methylene Chloride (1:1), (Supelco Analytical), containing the following PAHs: Naphtalene, Acenaphtylene, Acenaphtene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Dibenzo(a,h)anthracene, Benzo(g,h,i)perylene, and Indeno(1,2,3-cd)pyrene

Acetonitrile was LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak).

Chromatographic conditions

Parameter	Setting
Column:	Agilent ZORBAX Eclipse PAH 4.6 × 150 mm, 5 µm
Mobile phase:	A = Water, B = Acetonitrile
Gradient:	at 0 min 40% B
	at 12 min 80% B
	at 12.1 min 95% B
	at 25 min 95% B
Stop time:	25 min
Post time:	5 min
Flow rate:	1.5 mL/min
Injection volume:	Various
Column temperature:	25 °C
Variable wavelength detector:	230 nm

Results and discussion

Delay calibration

The delay calibration procedure determines the delay time between the detector and the fraction collector in the system. The delay time is used to compensate for the time it takes for the compound to travel between the point of **detection** in the detector and the point of **collection** in the fraction collector. With the Agilent 1260 Infinity Fraction Collector, the delay calibration procedure is done using the fraction delay sensor (FDS), a very simple detector built in into the fraction collector.

Together with the signal from the Agilent 1220 LC Systems' UV detector the signal from the FDS facilitates determination of the delay time between detector and fraction collector as shown in Figure 1. The delay calibration procedure is a completely automated process using the Agilent Lab Advisor Software. The result is a delay volume, which is then recalculated into a delay time for any given flow rate in the actual fraction collection method. For the system setup used in this Technical Overview, the delay volume of the system was determined to be 83 µL as shown in Figure 2. This delay volume is stored in the fraction collector configuration.

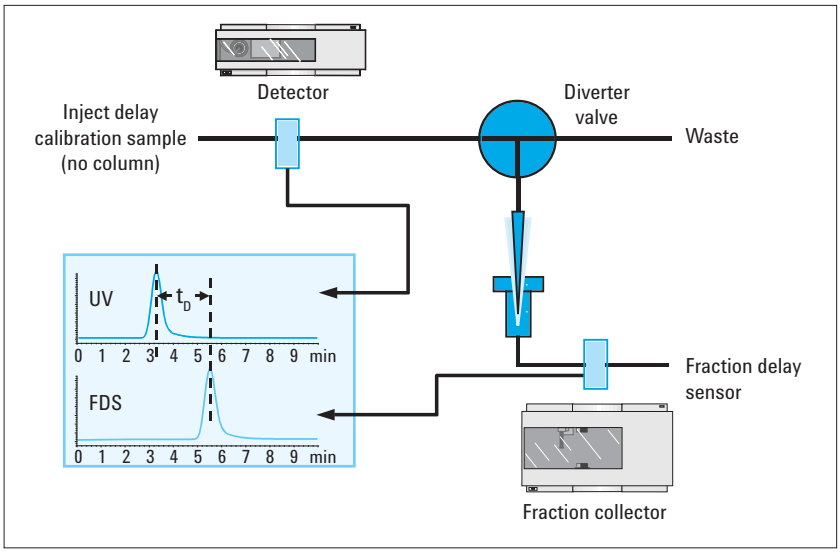


Figure 1
Delay calibration procedure.

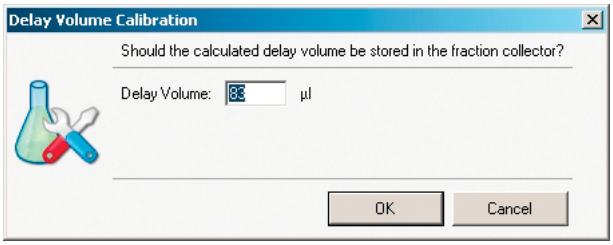


Figure 2
Result of the delay calibration procedure using the Agilent Lab Advisor Software.

Time-based fraction collection

Besides manually-triggered fraction collection, time-based fraction collection is the easiest method to collect fractions from a chromatographic run. In the experiment shown in Figure 3, fractions of 1 minute were taken between 6 and 16 minutes in the chromatogram.

Another possibility of time-based fraction collection with the Agilent 1260 Infinity Fraction Collector is to divide a time window into a certain number of equidistant fractions. Regardless of what mode of time-based fraction collection is applied, this triggering mode is very unspecific. In this example, this can be clearly seen when the collected fractions are re-analyzed. In the example from Figure 3, fraction number three contains only one, pure compound eluting at about 8.8 minutes (Figure 4a). Fractions number one and two (Figure 4b) contain both the compound eluting at 7.5 minutes. This is a typical problem when using time-based fraction collection, especially when retention times start to shift due to column aging.

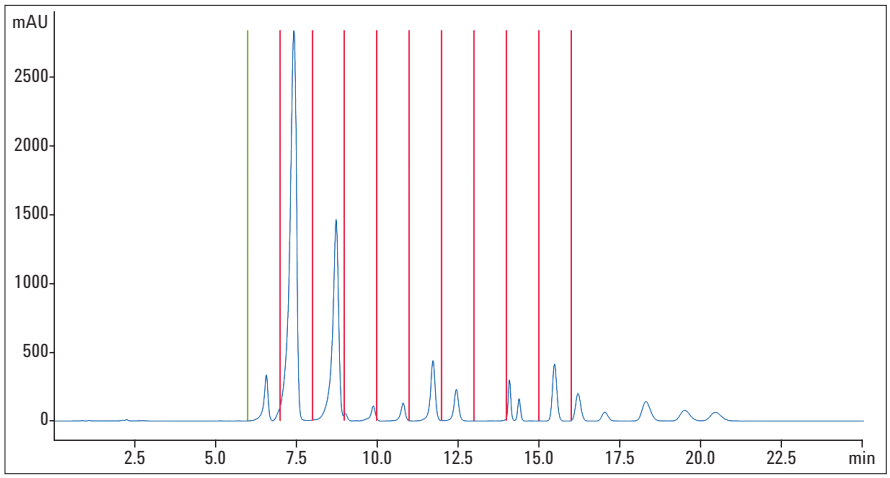


Figure 3
Result of time-based fraction collection.

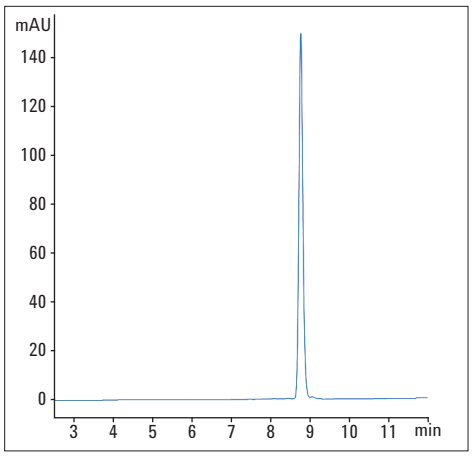


Figure 4a
Re-analysis of fraction 3.

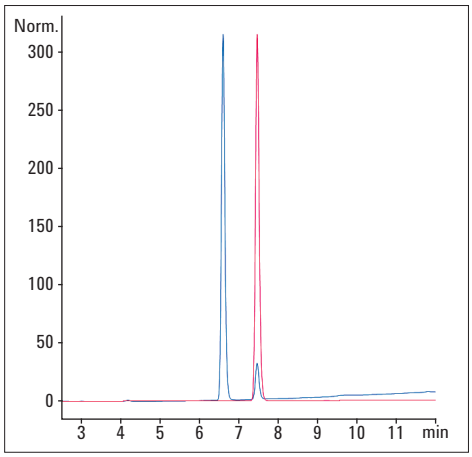


Figure 4b
Reanalysis of fractions 1 (blue) and 2 (red).

Peak-based fraction collection

Peak-based fraction collection is a more dedicated method to collect only the desired peaks and not unwanted baseline. To identify a peak and trigger the fraction collector, several parameters such as threshold, up slope, and down slope are available for the Agilent 1260 Infinity Fraction Collector[2]. In this experiment, simply a threshold of 500 mAU was set for peak identification. The resulting chromatogram and fraction collection results are shown in Figure 5.

As a result, only the major peaks are collected in dedicated fractions with high purity. This can be seen when re-analyzing the fractions, as shown for the first three fractions as an example in Figures 6 a–c.

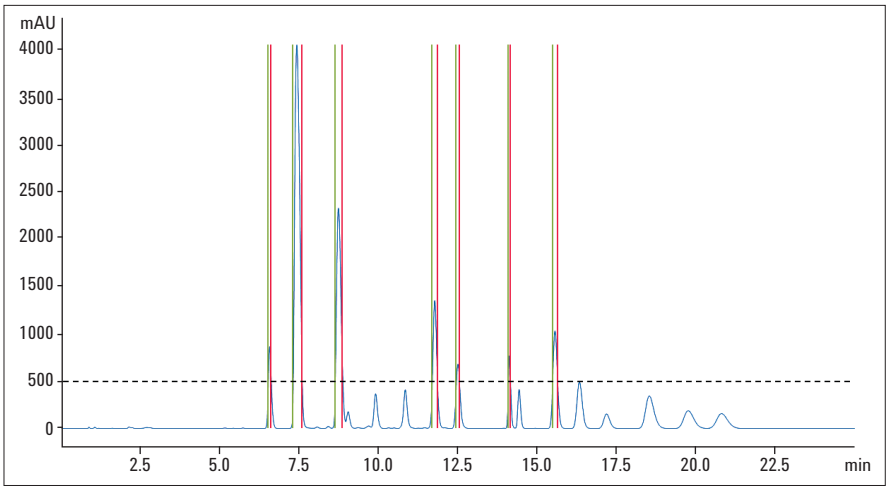
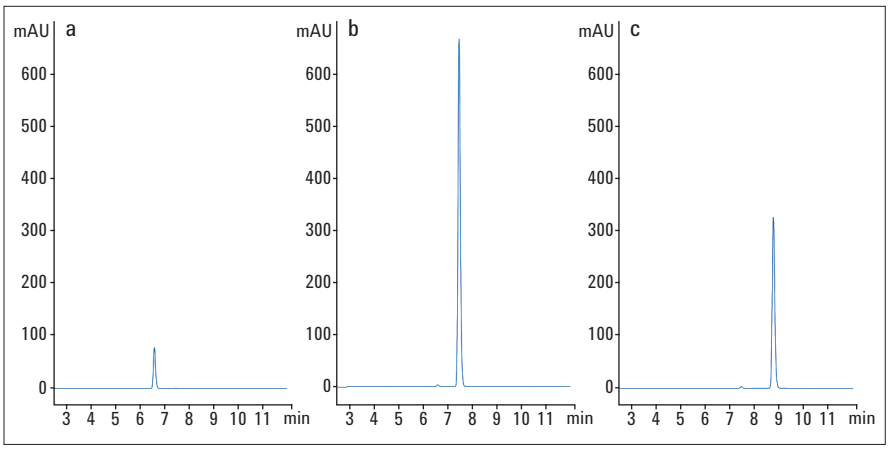


Figure 5
Peak-based fraction collection using a threshold of 500 mAU.



Figures 6 a –c
Re-analysis of fractions 1 – 3.

Conclusion

In this Technical Overview, we show the combination of the Agilent 1220 Infinity LC System and the Agilent 1260 Infinity LC Analytical-scale Fraction Collector as a simple and cost-effective system for analytical-scale preparative LC. Dedicated features such as the automated delay calibration procedure and the design of the fraction collector for lowest delay volumes, ensure infinitely better fraction collection leading to high purities and recoveries.

References

1.
"Principles in preparative HPLC" Agilent Technologies Primer, **2004**, publication number 5989-0652EN

2.
"Sophisticated peak-based fraction collection – working with up and down slope" Agilent Technologies Application Note, **2010**, publication number 5989-0511EN

Using the Agilent Instrument Control Framework to control the Agilent 1220 Infinity LC System through Waters Empower software

Instrument set up and performance

Technical Overview

Author

A.G.Huesgen

Agilent Technologies, Inc.

Waldbronn, Germany

Abstract

The Agilent Instrument Control Framework (ICF) enables other providers of LC data acquisition and processing software to simplify the development of the control of Agilent LC instrumentation. In this Technical Overview, we demonstrate how Agilent ICF facilitates enhanced control of the Agilent 1220 Infinity LC system through Waters Empower chromatography data software versions 2 and 3. The combination of Agilent ICF and Waters Empower software provides easy access to advanced features of the 1220 Infinity LC system such as overlapped injections.

Introduction

The Agilent Instrument Control Framework (ICF) is a software component that makes it easier and faster for software providers to implement control of Agilent LC equipment in their chromatographic data systems or workstations[1,2]. Based on new standard instrument drivers from Agilent, ICF eliminates much of the delay and effort of using low-level instrument control codes and the need of software developers to write their own native drivers.

In this Technical Overview, we demonstrate:

- What prerequisites have to be fulfilled to ensure seamless interaction between Agilent 1220 Infinity LC systems, Waters Empower software, and Agilent ICF
- How instruments are configured and methods are created for the Agilent 1220 Infinity LC system using Waters Empower software in combination with Agilent ICF
- That the performance of the Agilent 1220 Infinity LC system fulfills expectations in combination with Waters Empower data acquisition and processing tools

Experimental

An Agilent 1220 Infinity LC system with the following configuration was used for the precision measurement:

- Gradient pump
- Autosampler
- Column oven
- Variable wavelength detector
- Agilent ZORBAX RRHT Eclipse Plus C-18 column packed with 1.8-µm particles

Chromatographic conditions for precision measurement

Compounds:	RRLC Checkout sample (p/n 5188-6529), acetanilide, acetophenone, propiophenone, butyrophenone, benzophenone, valerophenone, hexanophenone, heptanophenone, octanophenone
Column:	Agilent ZORBAX RRHT Eclipse Plus C-18, 4.6 × 100 mm, 1.8 µm
Mobile phases:	A = Water, B = Acetonitrile
Gradient:	20% to 80% B in 8 min, at 9 min 80% B, at 9.01 min 20% B
Flow rate:	1.2 mL/min
Stop time:	12 min
Column temperature:	30 °C
Injection volume:	1 µL
VWD:	245, 10 Hz
Software:	Empower 2 Build 2154, Installed Service Packs: A-D
Installed feature releases:	1-4, with ICF version A.01.02 SP1

Prerequisites for the combination of Empower and ICF

- Only Agilent 1220 Infinity LC systems containing an autosampler are supported. Instruments with a manual injector are not supported by Empower 2 or 3.
- All Agilent LC modules must have firmware version A.06.32 or B.06.32 or B.06.41 or higher.
- All Agilent LC modules must have RC.Net drivers.
- Agilent Infinity LC ICS 1.0.0
- Empower 2, feature release 3 or higher or Empower 3 software[3]
- Windows XP with service pack 3 or higher[3]

Results and discussion

Agilent ICF facilitates access to advanced features of Agilent LC instruments that were previously not supported by earlier revisions of Empower with drivers provided by Waters. Now, all features are supported and available though the new *On line* screen, which has been added to the familiar Empower screen, see Figure 1. A right-click on one of the module fields gives access to all control, method and other advanced features of the module.

For supported modules and functions, refer to the Appendix.

Configuring the Agilent 1220 Infinity LC system

- Set up the DHCP of the Empower node.
- Set all DIP switches of the Agilent module to 0. This module is connected to the Empower node through LAN.
- The LC receives an IP address from the DHCP server.
- To connect the Agilent instrument, configure the DHCP server through *Properties* of the *Empower Nodes*.
- Use *Edit* to set the instrument type and a unique name.
- Click *File* and *New chromatographic system* to make the new LC system accessible for data acquisition.

After this last configuration step, the Agilent 1220 Infinity LC system is *On Line* and ready for use.

These configuration steps have to be followed whenever a new module is added or removed. Previously, the old configuration has to be deleted from the DHCP server configuration. Then the Empower software has to be shut down and the LAN connection to the module has to be switched off and on again. When the LAN connection has been restored, the new configuration procedure can be started.

Creating an instrument method and a method set

Having configured the instrument, the instrument method and the method set can be created in the *Empower Run* sample screen. The Instrument method is set up through the *Edit* method. All parameters that are available in the Agilent ChemStation are now typically accessible in Empower.

The created instrument method is saved and used to set up a method set. The method set can then be used to create sequences.

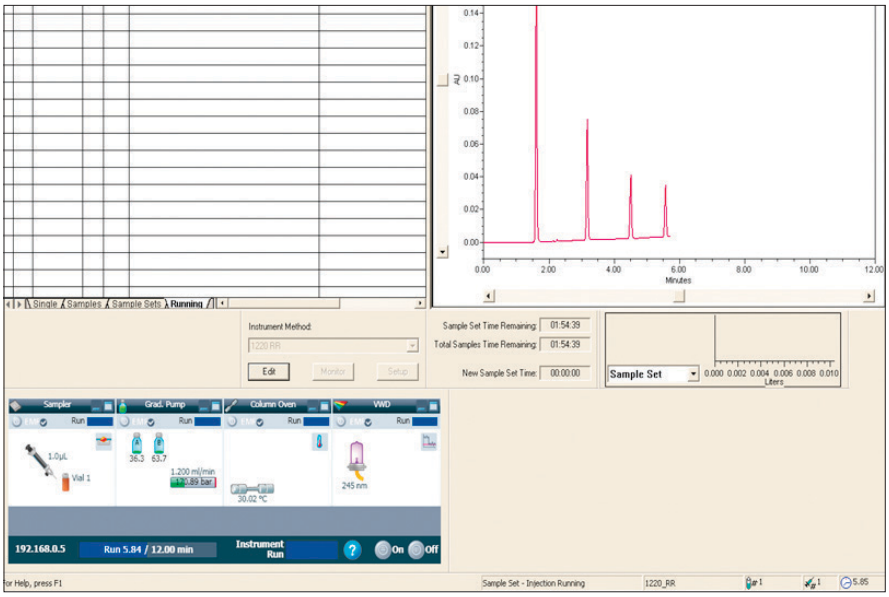


Figure 1
Agilent Instrument Status screen under Empower software and ICF, captured while a sequence was running.

Creating a sequence

A sequence is created by filling the sample set table with name of sample, position of vials, number of injection per vial, method set to be used, and so forth.

Performance of the Agilent LC systems using Waters Empower data processing tools

To demonstrate that the Agilent 1220 Infinity LC system fulfills the expected performance the following tests were done using the RRLC Checkout sample:

- Precision of retention time
- Precision of areas

Precision of retention time and areas

Precision of retention times and areas for a 1 µL injection are combined in Table 1. Data were evaluated using the Empower Component summary report. The precision for the retention times for six consecutive runs is less than 0.24% RSD, for the area the precision is less than 0.9% RSD.

Peak name	RSD RT (%)	RSD Area (%) (1 µL injection volume)
acetanilide	0.236	0.229
acetophenone	0.149	0.308
propiophenone	0.066	0.392
butyrophenone	0.034	0.518
benzophenone	0.041	0.334
valerophenone,	0.051	0.459
hexanophenone	0.050	0.510
heptanophenone	0.039	0.605
octanophenone	0.038	0.838

Table 1
Precision of retention times and areas for six consecutive runs.

Conclusion

The Agilent Instrument Control Framework (ICF) is a software component that makes it easier and faster for software providers to implement control of Agilent liquid chromatography systems in their chromatographic data systems or workstations. In our application example, ICF was used to control the Agilent 1220 Infinity LC system in combination with Waters Empower software. The instrument was configured in Empower and data were acquired and processed. The combination of ICF and Empower software facilitates access to all available Agilent instrument features such as overlapped injection. The Agilent Instrument Status screen is used to set up On Line methods, to switch the system on or off, to equilibrate columns, to view the status of single module, and to access special features using the Control function available for each Agilent LC module. As expected, the Agilent 1220 Infinity LC system shows the same excellent performance for data acquired and processed using Empower and ICF.

References

1.
"The Agilent Technologies Instrument Control Framework", Technical Overview, Publication number 5990-6504EN, November 2010

2.
"The Agilent Technologies Instrument Control Framework", Short overview, Publication number 5990-5756EN, June 2010

3.
"Agilent Infinity LC Instrument Component software Version 1.0 for Empower software", Waters Installation note, Publication number 716003453 Rev.A, August 2011

4.
"Using the Agilent Instrument Control Framework to control the Agilent 1260 Infinity LC through Waters Empower software- Instrument set up and performance", Agilent Publication, Publication number 5990-9092EN, November 2011

5.
"Using the Agilent Instrument Control Framework to control the Agilent 1290 Infinity LC through Waters Empower software - Instrument set up and performance", Agilent Publication, Publication number 5990-9093EN, November 2011

Appendix

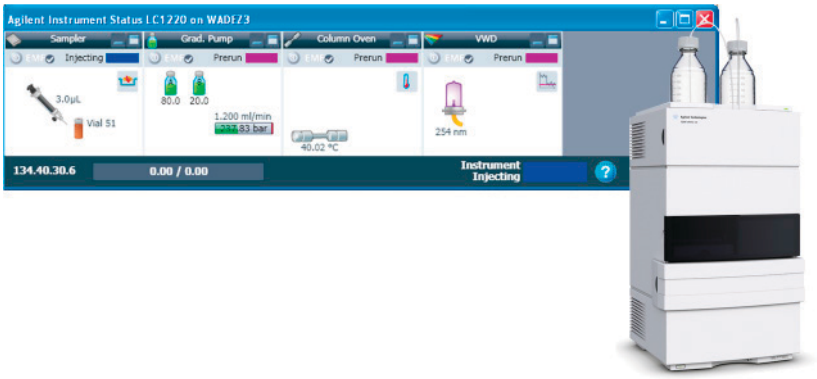
	Supported	Not supported
G4286B Isocratic, Manual Injector		X
G4288B/C Gradient, Manual Injector		X
G4290B/C Gradient, Autosampler	X	

Table 2
Supported and not supported instruments.

Using the Agilent Instrument Control Framework to control the Agilent 1220 Infinity LC System through Dionex Chromeleon software

Instrument set up and performance

Technical Overview



Author

A.G.Huesgen

Agilent Technologies, Inc.

Waldbronn, Germany

Abstract

The Agilent Instrument Control Framework (ICF) enables other providers of LC data acquisition and processing software to simplify the development of software control for Agilent LC instruments. Dionex Chromeleon 6.80SR11 A in combination with Agilent ICF provides enhanced control functions for all Agilent LC instruments. As an example, the Agilent 1220 Infinity LC System was connected to the new software features in the Dionex Chromeleon architecture. Nearly all Agilent 1220 Infinity LC instrument features are now accessible through the combination of Agilent ICF and Dionex Chromeleon software, such as overlapped injection.

Introduction

The Agilent Instrument Control Framework (ICF) is a software component that makes it easier and faster for software providers to implement control of Agilent LC equipment in their chromatographic data systems or workstations[1]. Based on new standard instrument drivers from Agilent, ICF eliminates much of the delay and effort of using low-level instrument control codes and the need for software developers to write their own native drivers [1, 2].

In this Technical Overview, the Agilent 1220 Infinity LC System was connected to Dionex Chromeleon and the functionality and performance was evaluated. The following is discussed:

- What prerequisites have to be fulfilled to ensure seamless interaction with 1220 Infinity LC Systems, Dionex Chromeleon software, and Agilent ICF
- How instrument methods and sequences for the 1220 Infinity LC system are set up in the Dionex Chromeleon software
- That the 1220 Infinity LC system also fulfills performance specifications under Dionex Chromeleon software
- Supported configurations are listed in the Appendix, Table 2

Experimental

Equipment

The instrument used was an Agilent 1220 Infinity LC System (G4290B), equipped with:

- Binary pump with vacuum degasser
- Autosampler
- Column oven
- Variable wavelength detector

Chromatographic conditions

Compounds:	Uracil, Phenol, methyl-, ethyl-, propyl-, butyl- and heptylparaben
Column:	Agilent ZORBAX Eclipse C18 RRHT, 4.6 × 100 mm, 1.8 µm
Mobile phases:	A=Water, B=Acetonitrile
Gradient:	20% B to 90% B in 8 min 20% B at 8.01 min
Flow rate:	1.2 mL/min
Stop time:	12 min
Column temperature:	40 °C
Injection volume:	3 µL
Detection:	254 nm, 20 Hz

The minimum prerequisites needed to run the Agilent 1220 Infinity LC with Dionex Chromeleon and Agilent ICF include:

- Dionex Chromeleon software version 6.80 SR11A
- Agilent ICF and Agilent LC Driver Package (version A.01.02 or higher) installed on the PC
- All hardware installed and instrument connected to the PC through LAN.

Results and discussion

With Agilent ICF, it is now possible to support Agilent LC instrument features which were not supported with previous Dionex Chromeleon software versions, using native Dionex drivers. Especially features available in the *On Line* screen which is added to the other Dionex Chromeleon screens, (Figure 1). Click the *ICF Status* window in the Dionex Chromeleon control panel screen to start the *1220 Infinity Status* window. A right mouse-click in one of the module windows gives access to all control, methods and other features of this module. For more detailed information, see[3,4].

The user interface is used for direct control of the 1220 Infinity LC System. Right mouse click in *Grad. Pump* and then select *Method* to open the Method screen for the pump. Flow rate and organic percentage can be selected to equilibrate the system. This is for control and equilibration only; no instrument method is created here.

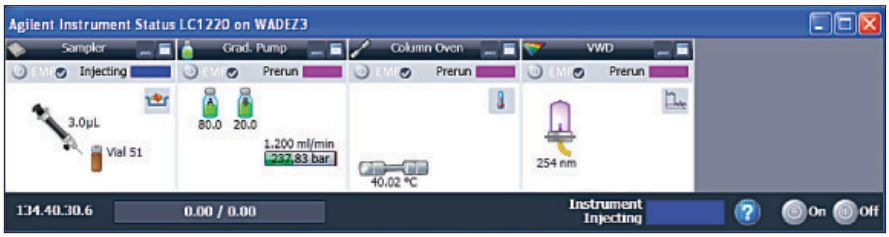


Figure 1
New Agilent Instrument Status screen under Dionex Chromeleon software and ICF software.

Creating a method

After configuration and integration of the Agilent LC system in the Dionex Chromeleon architecture, the instrument method can be set up by clicking *File*, *New* and *Program File*. New sequences, reports and so on are created here.

Click *Launch Agilent ICF IME* to start the 1220 Infinity LC system method setup screens for pump, autosampler, column oven, and VWD.

After selecting the appropriate method values for all parameters, store the Method (Program) file in an appropriate directory.

Creating a sequence

A sequence is created using the Sequence Wizard. The wizard leads the user through a dialog, for example, sample name, setting position of vials, number of injections per vial, instrument method, and other entries. To save the created sequence and start the sequence, click *Batch* and *Start*, (Figure 2). When all runs are done, the Status column shows *Finished* for all runs, and data processing can be done.

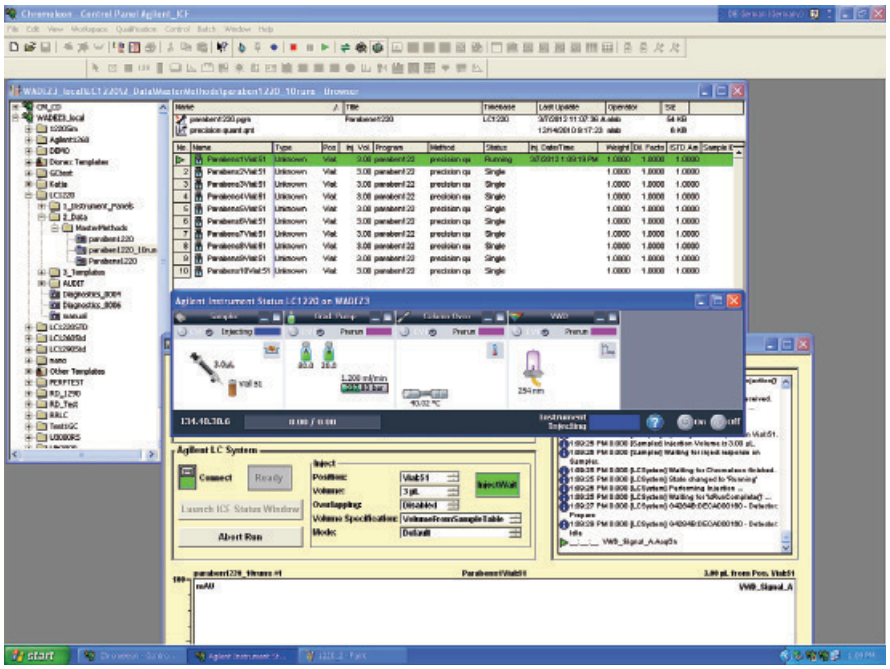


Figure 2
The Agilent 1220 Infinity LC system shown in the Dionex Chromeleon software, showing a sequence run.

Performance of Agilent LC systems using Dionex Chromeleon data processing tools

Precision of retention time and areas

Figure 3 shows the chromatogram of the paraben sample. Precision of retention times and areas for a 3-µL injection are combined in Table 1. Data were evaluated using the *Dionex Chromeleon Peak Summary* report. The precision for the retention times for six consecutive runs is <0.04% RSD, for the area the precision is <0.51% RSD. Both values are well within the specification limits for the 1220 Infinity LC System.

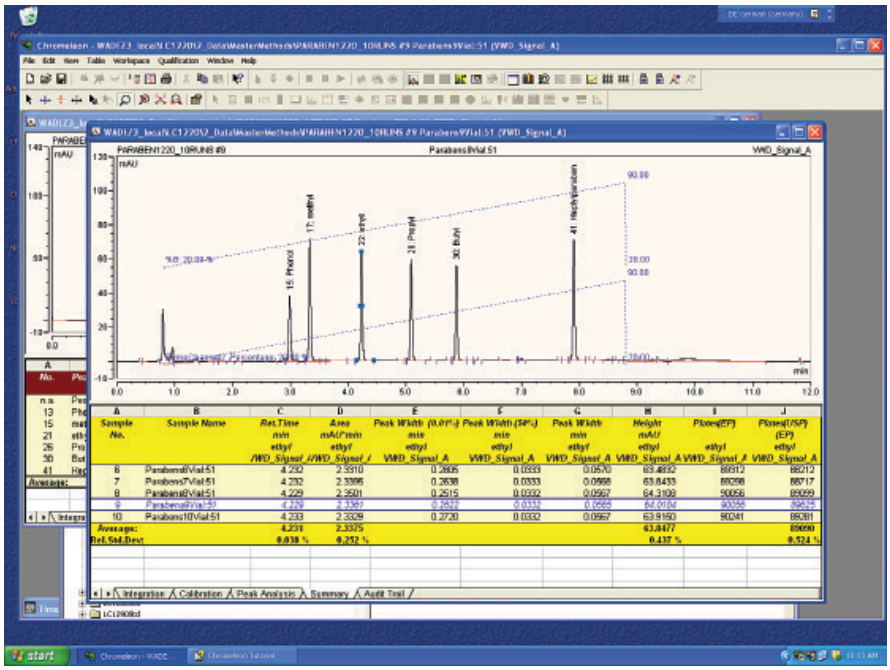


Figure 3
Chromatogram of the parabene sample for evaluation of retention time and area precision.

Conclusion

The Agilent Instrument Control Framework is a software component, that makes it faster and easier for other software providers to enable and control Agilent liquid chromatography systems through their chromatographic data systems or workstations. In our application example, the ICF software was used to control the Agilent 1220 Infinity LC System in combination with Dionex Chromeleon software. The instrument was configured in Dionex Chromeleon and data were acquired and processed. The combination of ICF software (version A.01.02 or higher) and Dionex Chromeleon 6.80 SR11A software allows access to nearly all Agilent instrument features such as injector programming. The Agilent instrument status screen is used to set up *On Line* methods, to switch the system on or off, to equilibrate columns, to view the status of single modules, and to access special features using the control function that is available for each Agilent LC module. As expected the 1220 Infinity LC System shows the same excellent performance for data acquired and processed using Dionex Chromeleon and ICF software.

Peak number	Peak name	RSD RT (%)	RSD Area (%) (3 µL injection volume)
1	Phenol	0.031	0.309
2	Methylparabene	0.029	0.182
3	Ethylparabene	0.038	0.252
4	Propylparabene	0.027	0.179
5	Butylparabene	0.024	0.203
6	Heptylparabene	0.013	0.501

Table 1
Precision of retention times and areas for six consecutive runs.

References

1.
"The Agilent Technologies Instrument Control Framework", Technical Overview, Publication number 5990-6504EN, **2010**.

2.
"The Agilent Technologies Instrument Control Framework" Short overview, Publication number 5990-5756EN, **2010**.

3.
Operation of the Agilent 1290 Infinity LC under Dionex Chromeleon software using Instrument Control Framework (ICF) *Instrument set up and performance,* Publication Number 5990-7215EN, **2011**.

4.
Operation of the Agilent 1260 Infinity LC under Dionex Chromeleon 6.8 software using the Agilent Instrument Control Framework (ICF), Publication Number 5990-7232EN, **2011**.

Appendix

	Supported
G4286B Isocratic, Manual Injector	x
G4288B/C Gradient, Manual Injector	x
G4290B/C Gradient, Autosampler	x

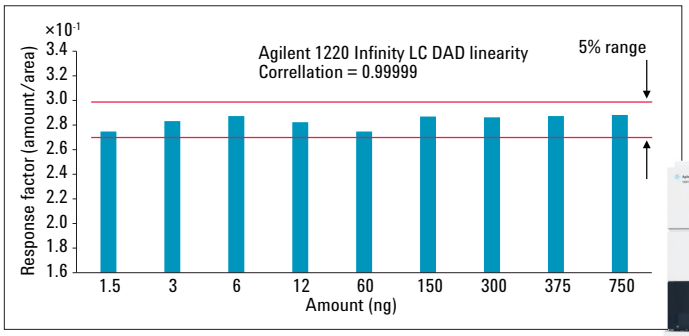
Table 2
Supported Agilent LC modules.

Performance of the Agilent 1220 Infinity Mobile LC

This section describes the performance characteristics of the Agilent 1220 Infinity Mobile LC. The 1220 Infinity LC Mobile Upgrade Kit consists of functional parts that enable the 1220 Infinity LC system to be mounted in a mobile laboratory vehicle so it can be moved to different locations to access remote measurement sites. The main component is the attenuation unit that acts as a shock absorber to protect the instrument during transit or from influences of operators moving in the mobile laboratory. A solvent bottle unit keeps the bottles fixed to the instrument. Wire mesh keeps the column safe in the column oven, and the mobile solvent compartment secures the solvent bottles during operation.

Performance Characteristics of the Agilent 1220 Infinity Gradient LC System with Diode Array Detector and Mobile Upgrade Kit

Technical Overview



Author

Sonja Schneider
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

The Agilent 1220 Infinity Gradient LC system is now available with a built-in diode array detector that offers a data acquisition rate of 80 Hz for full spectra. In combination with the Agilent 1220 Infinity Mobile Upgrade Kit, the system is resistant against shocks or vibrations during transportation in a mobile vehicle. The 1220 Infinity Mobile LC Solution is a robust and rugged system for onsite measurement. The performance of the system before and after vibration tests on a moving tray was evaluated with respect to retention time and area precision as well as noise and drift of the diode array detector. The performance of the diode array detector was analyzed in detail.

Introduction

The 1220 Infinity Gradient LC system is an integrated, binary-gradient liquid chromatography system with a pressure range of up to 600 bar, supporting both HPLC and UHPLC technology, including sub-2 µm and superficially porous columns. The system is available with a built-in diode array detector (DAD) for multiwavelength detection and spectra analysis. The DAD features a data acquisition rate 80 Hz, multiwavelength detection and spectral analysis. Other system modules include a dual-channel gradient pump, autosampler and column oven. The gradient pump has a flow rate range from 0.2 to 10 mL/min (5 mL at 600 bar, 10 mL at 200 bar), low pressure mixing and an integrated degassing unit. The autosampler operates with an injection volume range from 0.1 to 100 µL and a capacity of one hundred 2-mL vials. The column oven holds one 25-cm column, and the maximum temperature is 60 °C.

The 1220 Infinity LC Mobile Upgrade Kit consists of functional parts that enable the 1220 Infinity LC system to be mounted in a mobile laboratory vehicle so it can be moved to different locations to access remote measurement sites. The main component is the attenuation unit that acts as a shock absorber to protect the instrument during transit or from influences of operators moving in the mobile laboratory. A solvent bottle unit keeps the bottles fixed to the instrument. Wire mesh keeps the column safe in the column oven, and the mobile solvent compartment secures the solvent bottles during operation.

This Technical Overview shows that a wide range of parameters were tested with detailed DAD performance evaluation.

The pump and autosampler were tested for:

- Precision of retention times
- Precision of areas

The DAD was tested for:

- ASTM drift and noise for the 10-mm path length cell
- Linearity over a wide range
- Limit of detection for anthracene for the 10-mm path length cell
- Spectral performance

As the 1220 Infinity Gradient LC System with DAD is a mobile solution, the robustness and ruggedness was tested regarding pump, autosampler, and detector performance (noise and drift) before and after vibration tests on a moving tray.

Experimental

Instrumentation

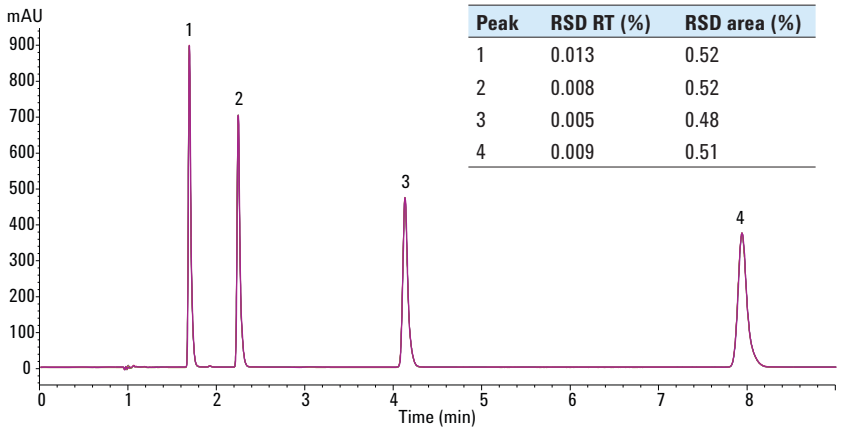
The Agilent 1220 Infinity Gradient LC System (G4294B) was equipped with a dual-channel gradient pump with integrated degassing unit, autosampler, column compartment, and the diode array detector. For transportation, the system was mounted on a transportation plate, 1220 Infinity Mobile Upgrade Kit (G4292A).

Software

- Agilent OpenLAB CDS ChemStation Edition for LC & LC MS Systems, Rev. C.01.04 [35]
- Agilent OpenLAB CDS 3D UV Add-On Software.

Pump and Autosampler Performance – Precision of Retention Times and Areas

Retention time precision was tested with different gradient and isocratic conditions using 4.6 and 3-mm id columns. The relative standard deviation (RSD) of retention times is typically < 0.2% for gradient analysis. Figure 1 shows an example of an isocratic application. The flow precision was < 0.015% RSD for the retention times.



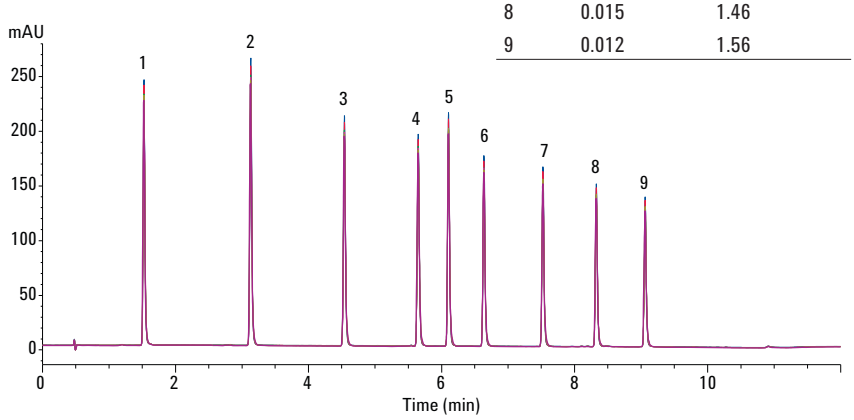
Peak	RSD RT (%)	RSD area (%)
1	0.013	0.52
2	0.008	0.52
3	0.005	0.48
4	0.009	0.51

Chromatographic conditions	
Sample:	Isocratic test sample (p/n 01080-68707)
Column:	Agilent ZORBAX RRHT, Eclipse Plus C18, 4.6 × 150 mm, 1.8 µm (p/n 959994-902)
Mobile phase:	A = Water B = Acetonitrile
Isocratic:	30/70 A/B
Flow rate:	1.2 mL/min
Stop time:	9 minutes
Injection volume:	5 µL, draw speed 200 µL/min
Column temperature:	40 °C
Detection:	254 nm/10 nm, Ref.: 360 nm/80 nm
Flow cell:	10 mm
Peak width:	> 0.025 minutes (0.5 second response time, 10 Hz)

Figure 1
Precision for an isocratic run at 230 bar with a retention time (RT) precision < 0.015% RSD.

Figure 2 shows an example of a gradient run. The retention time precision was < 0.063% RSD, except for the first peak.

Peak	RSD RT (%)	RSD area (%)
1	0.101	0.79
2	0.062	0.96
3	0.033	0.99
4	0.017	1.11
5	0.013	1.09
6	0.011	1.10
7	0.011	1.24
8	0.015	1.46
9	0.012	1.56

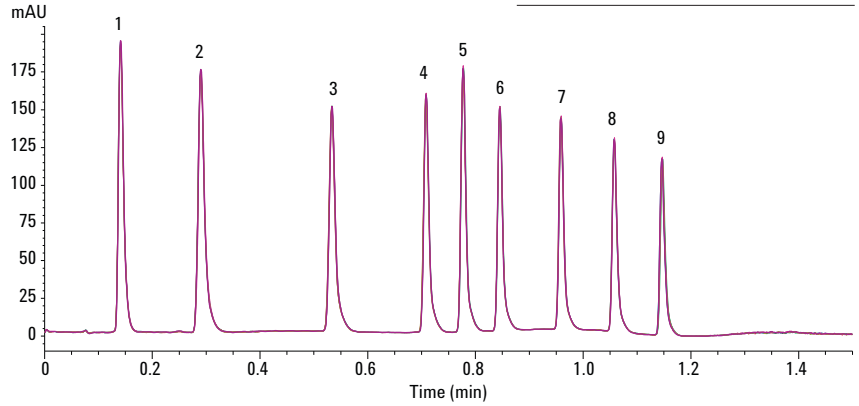


Chromatographic conditions	
Sample:	Agilent 1200 Series Rapid Resolution LC system checkout sample (p/n 5188-6529)
Column:	Agilent ZORBAX Solvent Saver HT, Eclipse Plus C18, 3.0 × 100 mm, 1.8 µm (p/n 959964-302)
Mobile phase:	A = Water B = Acetonitrile
Gradient:	20% B to 90% B in 10 minutes
Flow rate:	1.0 mL/min
Stop time:	12 minutes
Post-time:	5 minutes
Injection volume:	1 µL
Column temperature:	40 °C
Detection:	245 nm/10 nm, Ref.: 360 nm/80 nm
Flow cell:	10 mm
Peak width:	> 0.025 minutes (0.5 second response time, 10 Hz)

Figure 2
Precision for a gradient run at 434 bar with a RT precision < 0.063% RSD except for the first peak.

Figure 3 shows an example of a fast gradient run at 590 bar. In this experiment, an Agilent Poroshell 120 EC-C18 column was used. The retention time precision was < 0.08% RSD.

Peak	RSD RT (%)	RSD area (%)
1	0.045	0.86
2	0.078	0.43
3	0.056	0.51
4	0.028	0.45
5	0.028	0.74
6	0.023	0.48
7	0.037	0.66
8	0.016	0.73
9	0.048	0.77



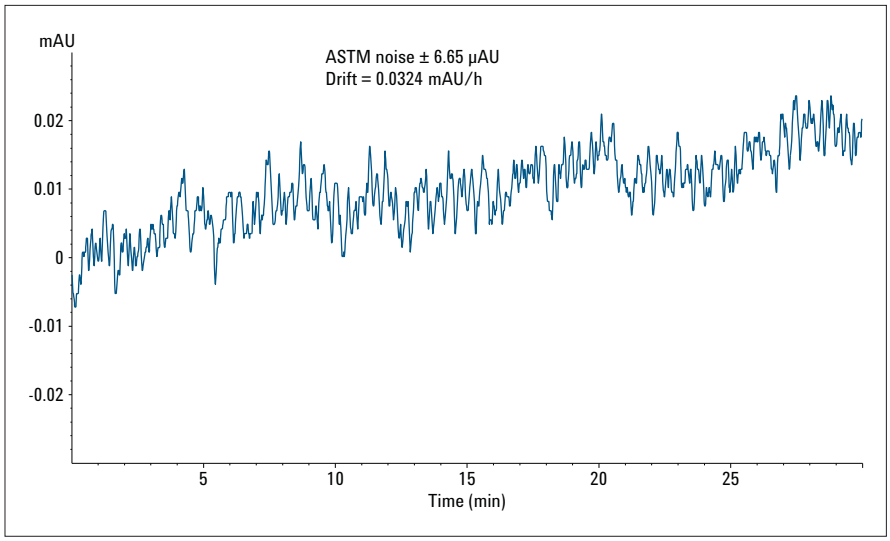
Chromatographic conditions	
Sample:	Agilent 1200 Series Rapid Resolution LC system checkout sample (p/n 5188-6529)
Column:	Agilent Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm (p/n 699975-302)
Mobile phase:	A = Water B = Acetonitrile
Gradient:	30% B to 95% B in 1 minute
Flow rate:	3.4 mL/min
Stop time:	1.5 minutes
Post-time:	1.0 minutes
Injection volume:	1 µL
Column temperature:	40 °C
Detection:	245 nm/10 nm, Ref.: 360 nm/80 nm
Flow cell:	10 mm
Peak width:	> 0.0063 minutes (0.13 second response time, 40 Hz)

Figure 3
Precision for a gradient run at 590 bar with a RT precision < 0.080% RSD.

Detector Performance

Noise and Drift

Evaluation of baseline noise was performed according to guidelines of the American Society for Testing and Materials (ASTM) in addition to drift measurements of the 10-mm path length flow cell. ASTM noise and drift was evaluated using a restriction capillary instead of a column and water as the mobile phase. The DAD was set to 1.25 Hz (4 second response). The resulting ASTM noise was ± 6.65 µAU and the drift was 0.0324 mAU.

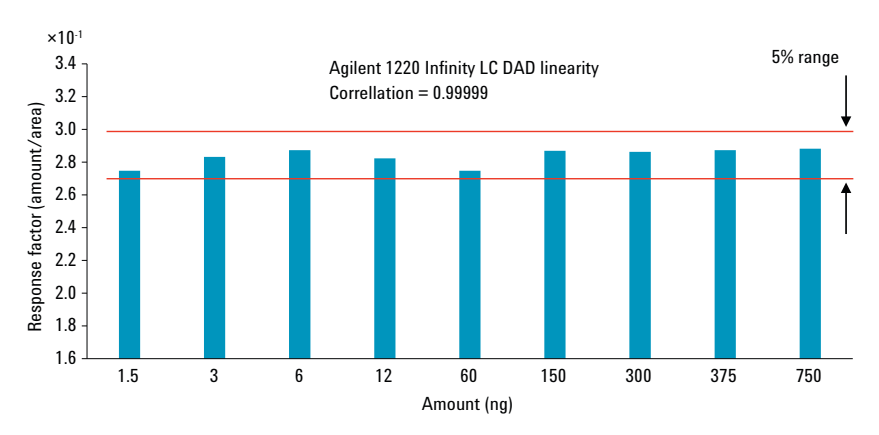


Chromatographic conditions	
Column:	Restriction capillary, backpressure 48 bar
Mobile phase:	Water, isocratic
Flow rate:	1.0 mL/min
Stop time:	30 minutes
Column temperature:	36 °C
Detection:	254 nm/10 nm, Ref.: 360 nm/80 nm
Flow cell:	10 mm
Peak width:	> 0.20 minutes (4.0 second response time, 1.25 Hz)

Figure 4
Noise and drift of the DAD.

Linearity for Different Caffeine Concentrations

Certified caffeine standards from 1.5 to 750 ng of injected amount were used to test detection linearity. Excellent linearity was obtained for this concentration range. The coefficient of correlation was 0.99999. The response factors were all within the ±5% error range from 1.5 to 750 ng, see Figure 5.

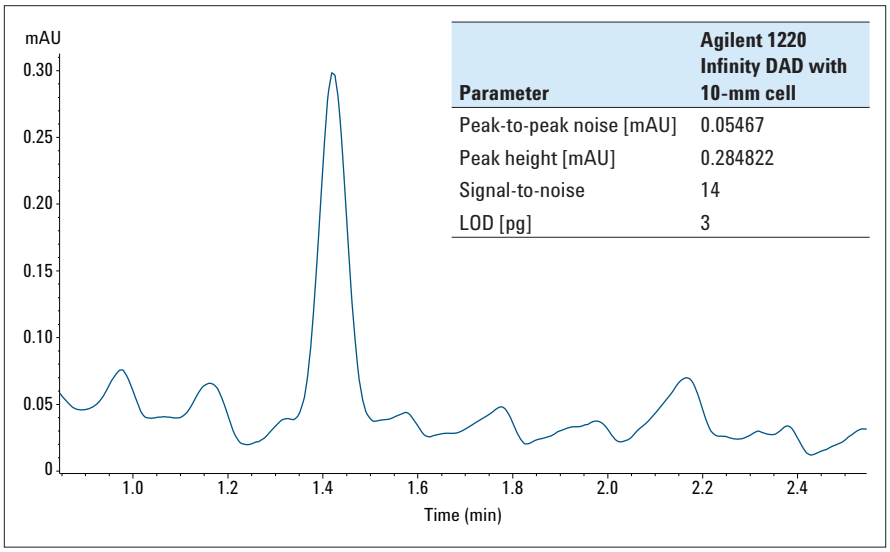


Chromatographic conditions	
Sample:	Certified caffeine standards
Column:	Agilent Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm (p/n 699975-302)
Mobile phase:	A = Water B = Acetonitrile
Isocratic:	90/10 A/B
Flow rate:	0.8 mL/min
Injection volume:	3 µL
Stop time:	1.5 minutes
Column temperature:	30 °C
Detection:	273 nm/10 nm, Ref.: 360 nm/80 nm
Flow cell:	10 mm
Peak width:	> 0.013 minutes (0.25 second response time, 20 Hz)

Figure 5
Linearity using certified caffeine standards as sample compound.

Limit of Detection for Anthracene

The limit of detection (LOD) for anthracene was evaluated using the DAD at 2.5 Hz. The injected concentration was as low as 5 pg/µL, see Figure 6. The LOD for a signal-to-noise ratio of 3 was 3 pg.



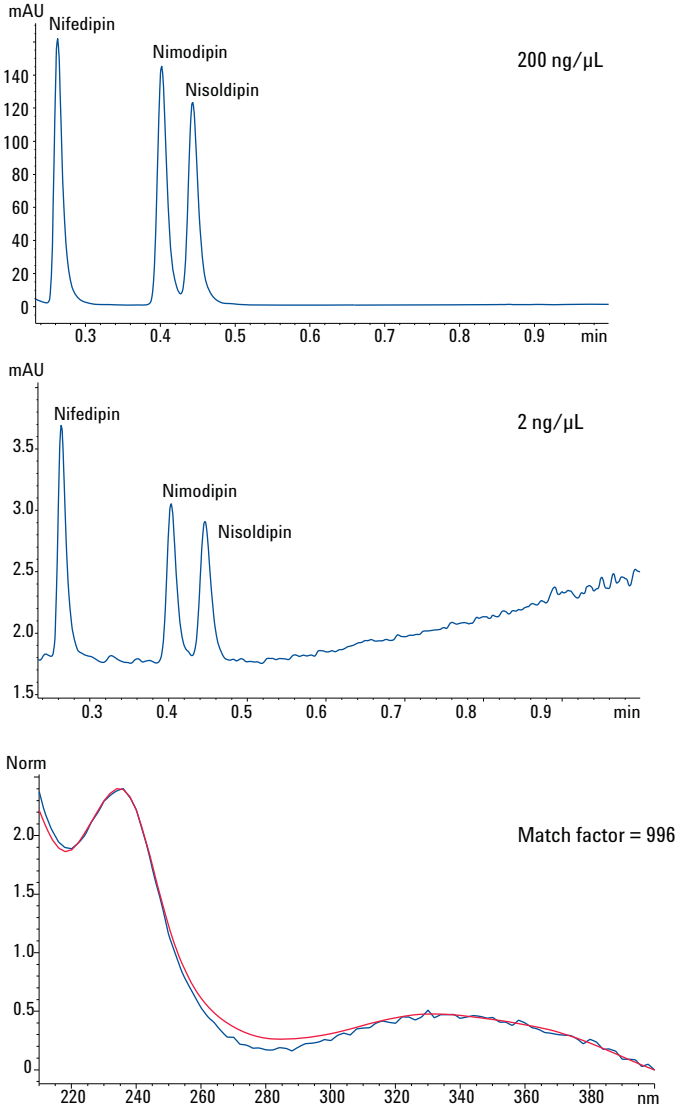
Parameter	Agilent 1220 Infinity DAD with 10-mm cell
Peak-to-peak noise [mAU]	0.05467
Peak height [mAU]	0.284822
Signal-to-noise	14
LOD [pg]	3

Chromatographic conditions	
Sample:	Anthracene, 5 pg in 1 µL
Column:	Agilent ZORBAX RRHD SB C18, 2.1 × 50 mm, 1.8 µm (p/n 857700-902)
Mobile phase:	A = Water B = Acetonitrile
Isocratic:	35/65 A/B
Flow rate:	0.5 mL/min
Stop time:	3.0 minutes
Column temperature:	36 °C
Detection:	251 nm/4 nm, Ref.: 450 nm/80 nm, 8 mm slit width
Flow cell:	10 mm
Peak width:	> 0.10 minutes (2.0 second response time, 2.5 Hz)

Figure 6
Limit of detection for anthracene.

Spectral Conformation of Trace-level Compounds Using Ultrafast Chromatographic Conditions

A library search was performed for the measured trace-level spectrum. Match factors were calculated and tabulated in the library research table. Highest spectral match was achieved for nifedipin with a match factor of 996. The spectral library analysis confirmed the compound identification based on chromatographic retention. This positive spectral confirmation significantly enhances confidence in qualitative analytical results, see Figure 7.



Chromatographic conditions	
Sample:	Nifedipin, nimodipin, and nisoldipin each 200 ng/µL and 2 ng/µL
Column:	Agilent ZORBAX RRHT SB C18, 4.6 × 50 mm, 1.8 µm (p/n 827975-902)
Mobile phase:	A = Water + 0.05% TFA B = Acetonitrile + 0.045% TFA
Gradient:	65% B to 70% B in 0.85 minutes
Flow rate:	3.0 mL/min
Injection volume:	1 µL, needle cleaning with methanol
Column temperature:	36 °C
Detection:	254 nm/4 nm, Ref.: 450 nm/80 nm, 8 mm slit width, all spectra
Stop time:	1 minute
Post time:	1 minute

Figure 7
Analysis of nifedipin, nimodipin, and nisoldipin for spectral and purity evaluation.

Peak Purity Analysis of Trace-level Compounds Using Ultrafast Chromatographic Conditions

The 1220 Infinity Gradient LC System with DAD enables peak purity analysis under ultrafast LC conditions, even for trace-level compounds. The spectral analysis confirmed that the nifedipin peak, as identified by chromatographic retention, was pure. This positive purity confirmation significantly increases confidence in quantitative chromatographic results, see Figure 8.

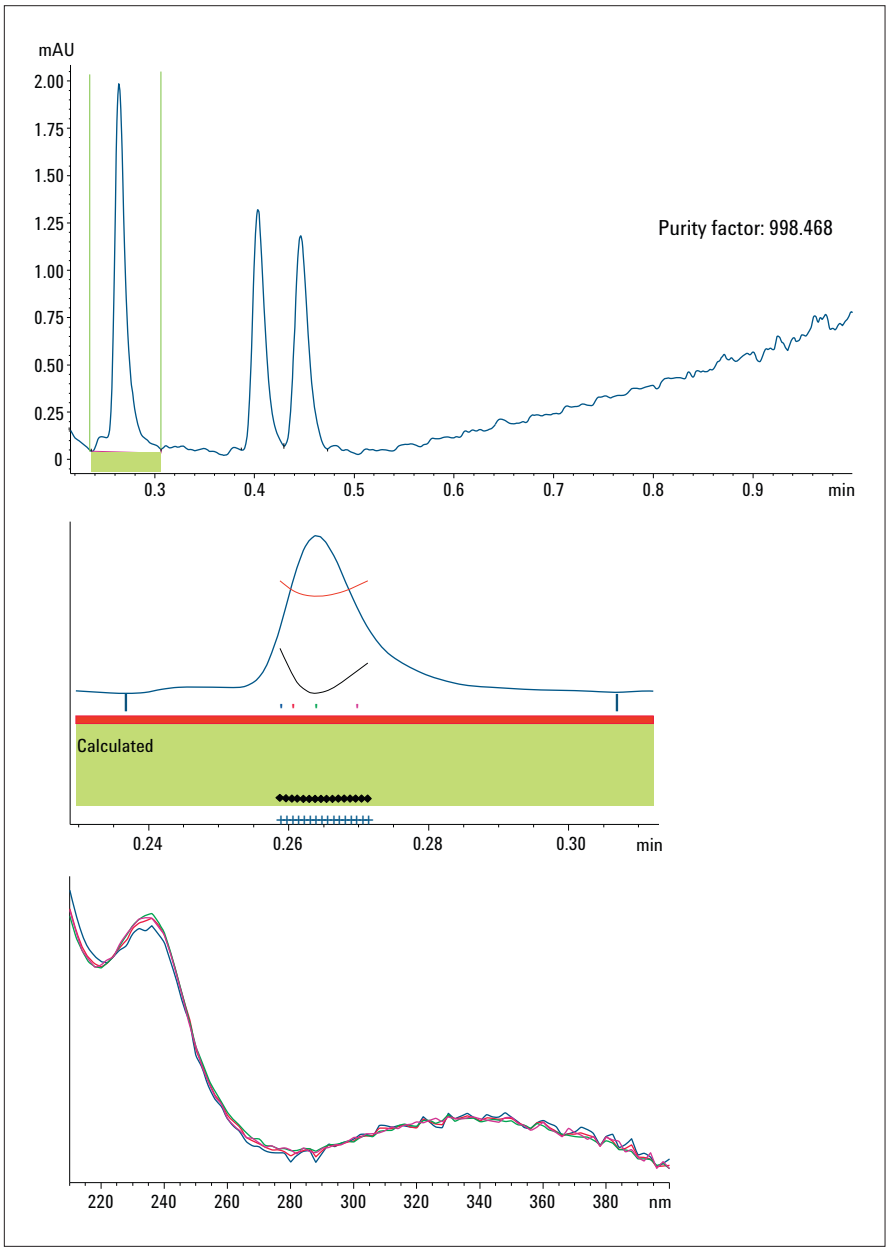


Figure 8
Analysis of nifedipin, nimodipin, and nisoldipin for spectral and purity evaluation.

Performance After Vibration Tests

To prove the robustness and ruggedness of the 1220 Infinity Gradient LC System with a DAD as a mobile solution, the system was subject to vibration tests on a moving tray. After the vibration tests, pump and autosampler performance was repeated as well as noise and drift measurement of the DAD.

Table 1 shows the results for the RSD values with respect to retention time and area before and after the vibration test. The values are all in a similar range before and after the test, resulting in the conclusion that the vibration did not affect pump and autosampler performance referring to RSD of retention times and areas.

Table 2 shows the results of the detector performance tests including the noise and drift measurements before and after the vibration tests. No major differences were found between the analysis before and after vibration.

Conclusions

The Agilent 1220 Infinity Gradient LC system is now equipped with a DAD for multiwavelength detection and spectra analysis. This Technical Overview shows that the performance of the 1220 Infinity LC system with a DAD meets the requirements of modern analytical liquid chromatography. The 1220 Infinity Gradient LC system with a DAD is a mobile solution for onsite measurement, which is proved by the reproducibility of very good RSD values for retention time and area before and after vibration tests. In addition, noise and drift measurements gave very similar results before and after vibration, showing that the 1220 Infinity Gradient LC system is a robust and rugged system for onsite measurement. The detailed performance analysis of the DAD revealed high linearity and sensitivity with optional spectral confirmation analysis.

	% RSD RT		% RSD area	
Experiment	**Test**	**After test**	**Before test**	**After test**
Long gradient run	< 0.063	< 0.069	< 1.6	< 1.0
Short gradient run	< 0.08	< 0.125	< 0.9	< 0.6
Isocratic run	< 0.014	< 0.011	< 0.055	< 0.045

Table 1
RSD of RT and area before and after vibration tests.

Experiment	Result
Noise	< 10 µAU (before and after vibration test)
Drift	< 0.04 (before and after vibration test)
Detector linearity	0.99999
LOD anthracene	3 pg

Table 2
Detector performance.

Environmental Applications

Today, environmental analysis must be done more reliably, more efficiently, and with even higher quality results than ever before. This application area covers the measurement of organic and inorganic chemicals in water, soil and textiles. The 1220 Infinity LC exhibits high robustness and flexibility for these applications.

Screening for EU banned dyes in textiles using the Agilent 1120 Compact LC with an Agilent 6140 Single Quadrupole LC/MS system and the Analytical Studio Browser software

Increased productivity by quickly identifying samples that fail regulatory requirements

Application Note

Environmental

Author

Syed Salman Lateef

Agilent Technologies

Bangalore, India

Abstract

The European commission has restricted the commercialization of textiles containing certain types of azo dyes. The azo bond (-N=N-) in the dyes can undergo reductive cleavage to produce by-products such as aromatic amines, some of which are potential carcinogens. Twenty-two aromatic amines are classified by European directives as carcinogens whose concentration in textiles should not exceed 30 ppm for each amine. In this work, a literature method has been modified to include MS-compatible buffers for analysis of EU banned dyes in textiles. Analysis was performed using an Agilent 1120 Compact LC coupled to an Agilent 6140 Single Quadrupole LC/MS system and Agilent Analytical Studio Browser (ASB) software. ASB allows quick visual identification of samples that exceed regulatory requirements. Such a setup is beneficial for textile testing laboratories where large numbers of textile samples are tested for qualification.

Introduction

The EU has listed 22 aromatic amines as potential carcinogens and maintains an acceptable upper limit of 30 ppm for each in textiles. This testing method involves the reduction of dyes in textiles with reducing agents, followed by the extraction of by-products. The by-products are matched with the list of 22 restricted amines and their concentration determined. In addition to official methods, there are other methods reported in literature that maximize the recovery. A modified literature method[1] that uses MS-compatible buffers during reduction and extraction is shown here.

An Agilent 1120 Compact LC, with the Agilent 6140 Single Quadrupole Mass Spectrometer is operated by ChemStation B.04.02.

Analytical Studio Browser (ASB) is a visual tool that allows rapid identification of samples exceeding threshold levels set by the analyst. It is an add-on software to ChemStation. The main features of ASB provide the user with the following capabilities:

- Browse very large amounts of LC/MS data very quickly.
- Assess the quality of data taken from a variety of detectors.
- Edit data and override data processing decisions made by automated systems.
- Report the data in a format that fits the particular needs of their work environment.

The methodology can be extended to analysis of other consumer samples such as toys or food products where a simple visual assessment of pass or fail is required.

Experimental

The standards of 22 restricted aromatic amines were purchased from Sigma Aldrich. Four colored polyester textile samples were purchased from local stores in India for analysis.

Twenty-two aromatic amines standard stock solution

The 22 aromatic amine standards were dissolved in acetonitrile (90:10): 25 mM ammonium acetate solution to a concentration ~3000 ppm (100% methanol also can be used). The solution was further diluted to 100-ppm solutions. The 100-ppm solutions were further diluted in 10% mobile phase B and 90% mobile phase A to 1-ppm solutions for determining the fragmentor voltage.

Textile sample

A 1.0-mL amount of freshly prepared, 1M aqueous ammonium hydroxide containing 50 mg of sodium dithionite was added to 0.1 g of shredded textile samples and heated at 80 °C for 90 min. Next, 1 mL of 100% mobile phase B was added to the textile sample and microwaved (1350 W) for 10 sec, then pipetted out. The procedure was repeated twice, using 10% mobile phase B and 90% mobile phase A as the extracting solvent. The extracted solutions were combined and 100 µL of formic acid were added to neutralize the pH. The solution was diluted to the 5-mL mark with water. An EU upper limit content of 30 ppm in 0.1 g of textile corresponds to 3 µg in 5 mL. The solution was syringe-filtered with a 0.45 µm filter before analysis.

Aqueous linearity samples

Twenty-two restricted amines were prepared to concentrations: 9 µg/5 mL, 6 µg/5 mL, 3 µg/5 mL, 1 µg/5 mL, 0.6 µg/5 mL in 5% mobile phase B.

Experimental Parameters	Details	
Column	Agilent ZORBAX Eclipse Plus C18, 150 mm × 3.0 mm, 3.5 µm p/n 959963-302; operated at 30 °C	
Mobile phase	Buffer A: Water 0.1% formic acid Buffer B: Methanol with 0.1% formic acid	
Gradient run	Run time (min): 42 min 8.8% B – 0 min 10% B – 10 min 16% B – 10.1 min 22% B – 20 min 53% B – 20.1 min 62% B – 30 min 100% B – 30.5 min 100% B – 35 min 8.8% B – 35.1 min 8.8% B – 42 min	
Flow	0.7 mL/min	
Injection volume	5 µL	
Variable wavelength detection (VWD)	254 nm	
6140 MSD parameters ESI Source: Positive mode SIM mode, peak width 0.03 min	Drying gas	13.0 L/min
	Nebulizer pressure	40 psig
	Dry gas temperature	350 °C
	Capillary voltage	4000 V
ASB parameters (B.02.00)	TIC – integration set to off at all time points BPI – 40%	

Positive control

Acid red 4 (control 1) and direct blue 15 dyes (control 2) were dissolved in methanol to dye polyester textiles. A 0.1-g sample of dyed textile was used as a positive control.

Results and discussion

Method development and analysis of a standards mixture

A mixture of 22 aromatic amines (each 1 ppm) was analyzed using VWD and MSD in series. A linear water-methanol gradient of 10% to 90% B with a 150 mm Agilent ZORBAX Eclipse Plus C18 column resolved most of the peaks well, compared to a phenyl column. In addition, methanol proved to be better than acetonitrile or a mixture of methanol and acetonitrile on a ZORBAX Eclipse Plus C18 column. A flatter gradient in combination with 30 °C column oven temperature was used to resolve overlapping peaks (9 and 10, 14 and 15). The specificity of the method was increased by operating the MSD in time-programmed SIM mode. Here, four time groups were added in data acquisition: 0 – 5 min, 5 – 10 min, 10 – 20 min and 20 min – 42 min to contain specific molecular ions in that time segment, thereby increasing the dwell time (Table 1). Figure 1 shows the MS total ion chromatogram and UV chromatogram for the standard mix (3 µg/5 mL) of 22 restricted amines. UV based detection of 22 restricted amines shows a varying response to 254 nm. The advantage of MS-based detection is increased sensitivity and selectivity compared to UV-based detection.

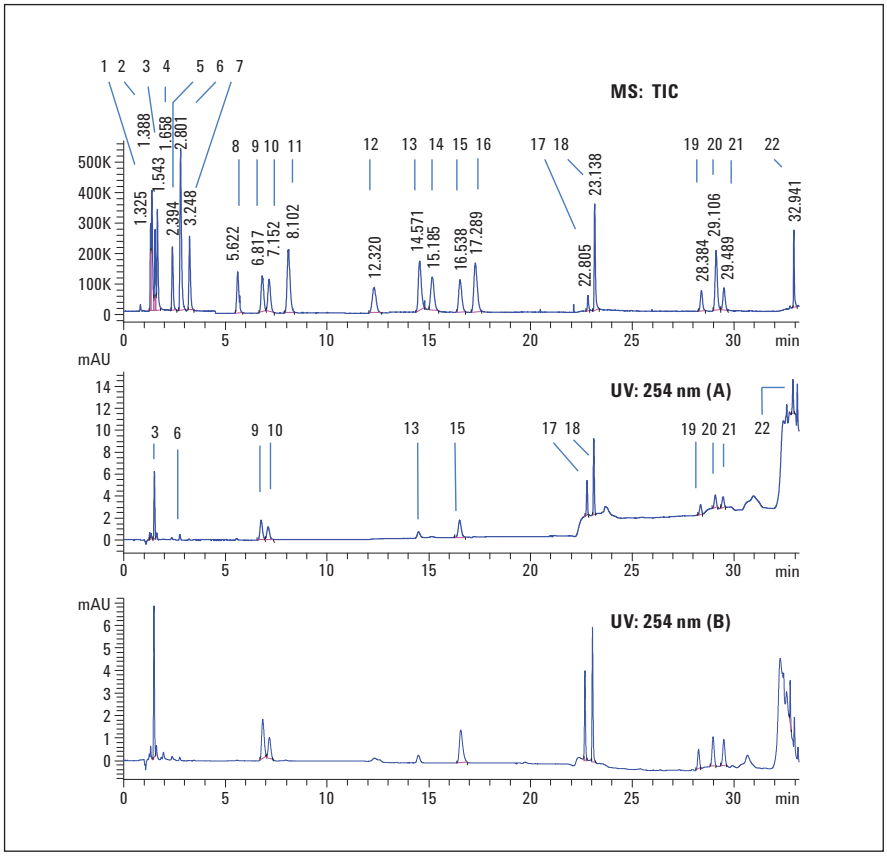


Figure 1
Total ion chromatogram (TIC) of the mixture of 22 aromatic amines operated in time-programmed SIM mode. UV detection at 254 shows varying responses to different aromatic amines. UV chromatogram (A) shows baseline shifts due to a rapidly changing gradient; UV chromatogram (B) shows the UV baseline is relatively straight when formic acid is not added in mobile phase B.

Compound name (based on time segments)	Abbreviated compound name	Molecular ion $(M+H)^+$	Fragmentor voltage (V)	Retention time (min)
Time Segment: 0 – 5 min				
4-Methoxy-*m*-phenylenediamine	1	139.1	82	1.3
2,4-Diaminotoluene	2	123.1	60	1.4
Benzidine	3	185.1	98	1.5
4,4′ -Oxydianiline	4	201.1	134	1.7
4,4′ -Diaminodiphenylmethane	5	199.1	108	2.4
o-Anisidine (2-Methoxyaniline)	6	124.1	78	2.8
o-Toluidine	7	108.1	100	3.2
Time Segment: 5 – 10 min				
4-Chloroaniline	8	128.1	110	5.6
o-Tolidine	9	213.1	112	6.8
o-Dianisidine (3,3′-Dimethoxybenzidine)	10	245.1	90	7.2
2-Methoxy-5-methylaniline	11	138.1	90	8.1
Time Segment: 10 – 20 min				
4,4′-Methylene-bis(2-methylaniline)	12	227.3	128	12.3
2-Naphthylamine	13	144.1	92	14.6
4-Chloro-2-methylaniline	14	142.1	102	15.2
4,4′-Diaminodiphenyl sulfide	15	217.1	130	16.5
2,4,5-Trimethylaniline solution	16	136.1	112	17.3
Time Segment: >20 min				
2-Methyl-5-nitroaniline	17	153.1	110	22.8
4-Aminobiphenyl	18	170.1	138	23.1
3,3′-Dichlorobenzidine solution	19	253	114	28.4
4-Aminoazobenzene	20	198.1	98	29.1
4,4′-Methylene-bis(2-chloroaniline)	21	267	144	29.5
Fast Garnet GBC base	22	226.1	94	32.9

Table 1
Fragmentor voltage and time segments used in data acquisition of EU-banned amines.

The precision of the method is demonstrated in Table 2, using six replicates of 3 µg/5 mL solution. The results show the relative standard deviation (RSD) for the retention time to be less than 0.1 min and the RSD for area response to be less than 5.6. The linearity at five concentration levels of extraction ion chromatograms show the correlation coefficient (R^2) to be greater than 0.99.

Abbreviated Compound Name	RSD of RT, n=6	RSD of Peak Area, n=6	Correlation Coefficient R^2
1	0.06	2.3	0.992
2	0.13	3.2	0.998
3	0.10	1.5	0.999
4	0.08	2.1	0.999
5	0.15	1.9	0.999
6	0.03	2.7	0.999
7	0.07	5.6	0.997
8	0.03	2.6	0.999
9	0.06	3.3	0.999
10	0.04	1.8	0.999
11	0.08	2.6	0.999
12	0.11	3.4	0.999
13	0.03	4.9	0.999
14	0.06	5.0	0.998
15	0.03	2.9	0.999
16	0.04	4.8	0.999
17	0.01	2.8	0.999
18	0.01	2.6	0.999
19	0.01	2.4	0.999
20	0.02	2.0	0.999
21	0.02	2.6	0.999
22	0.02	4.2	0.997

Table 2
The relative standard deviation (RSD) of retention time (RT) and peak area of all 22 restricted aromatic amines using six replicates of 3 µg/5 mL solutions. Correlation coefficient (R^2) value is for aqueous linearity samples.

Easy assessment of results using Analytical Studio Browser

ASB displays the results of the analysis and correlates them to the sample location in the autosampler. The integration events parameters define the peaks that are integrated. Only the integrated peaks and the percentage of base peak intensity (BPI) are used in ASB calculations to show the presence or absence of the compound in a particular sample.

Aromatic amine standards (3 µg/5 mL) were injected to determine the retention time (RT) and area. Using this database, any compound whose *m/z*, RT and area are within the acceptable range is integrated and shows as a target compound (Figure 2).

Azo dyes such as acid red 4 and direct blue 15 are known to degrade into banned aromatic amines: 2-Methoxyaniline and 3,3′-dimethoxybenzidine respectively[2]. Two polyester textiles were dyed separately using acid red 4 and direct blue 15. As shown in Figure 2, the textile sample dyed with acid red 4 produced *m/z* of 124.1 (Compound 6; 2-methoxyaniline). Similarly, the textile sample dyed with direct blue 15 produced *m/z* of 245.1 (Compound 10).

Four colored polyester textile samples were also analyzed but they did not yield any banned aromatic amines. If banned amines had been detected then the analyst could quantitate only those aromatic amines that are identified as the target. The results show that ASB provides confirmation of the presence or absence of specific banned amines; thereby eliminating the need to quantify all the 22 banned amines.

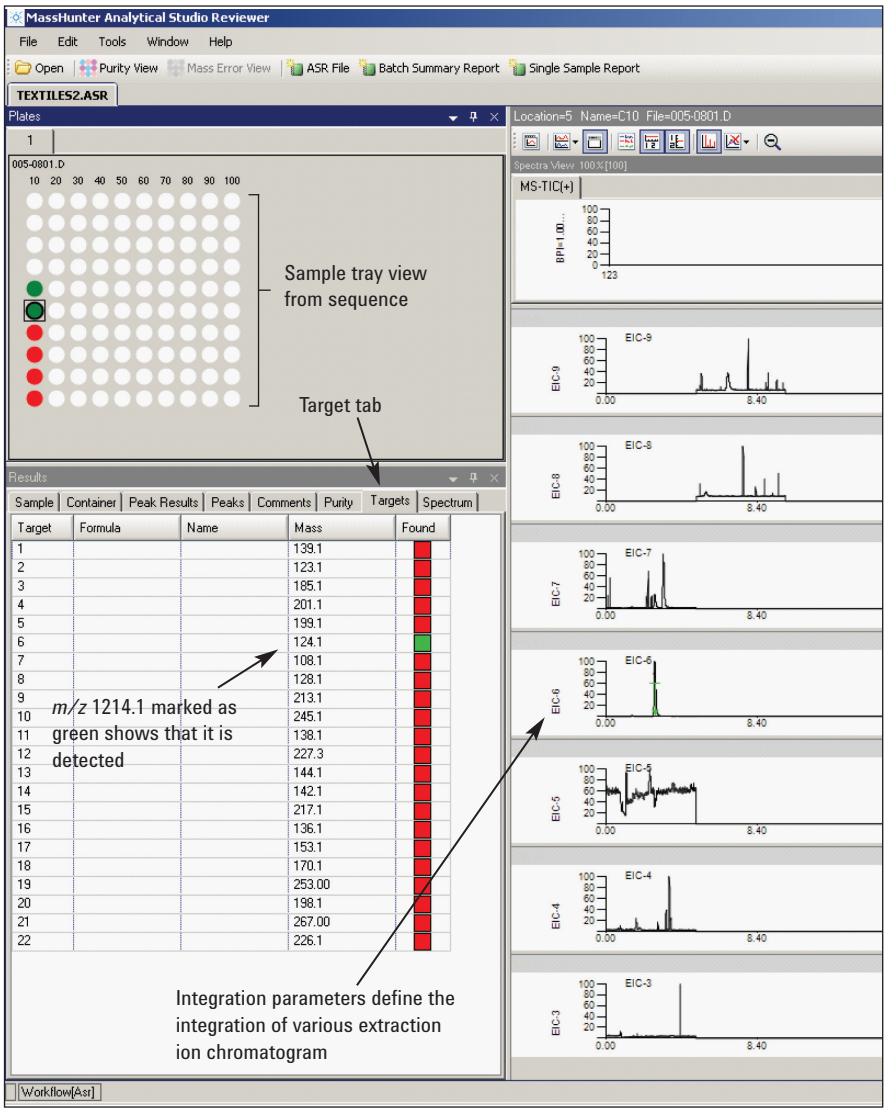


Figure 2
Screen Shot of Analytical Studio Browser showing a textile sample dyed with acid red 4 (control 1, sample position 20). ASB target list identifies 124.1 as compound detected.

Conclusion

The combination of the Agilent 6140 Single Quadrupole LC/MS system with the Analytical Studio Browser software provides a versatile tool for quick screening of textiles for compliance with regulatory standards. Quick identification of failed samples leads to reduced analysis time. Analytical Studio Browser provides visualization of samples that fail requirements.

References

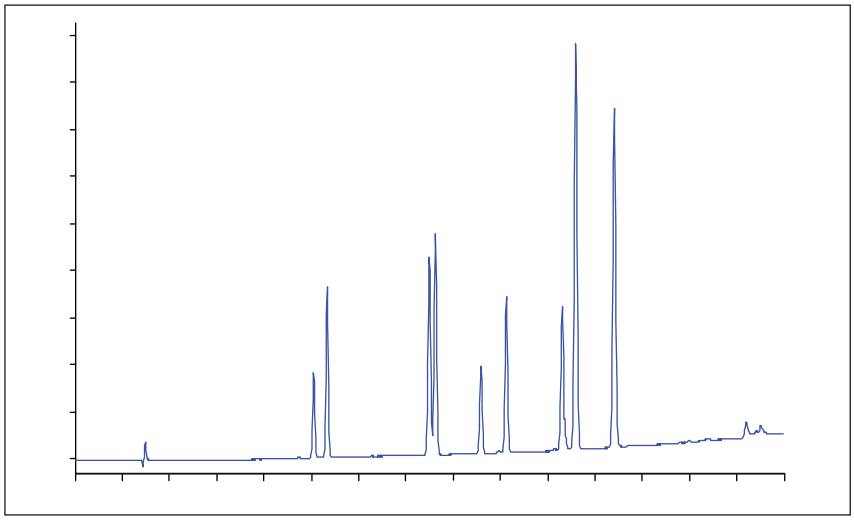
1. L. H. Ahlstrom, J. Raab, L. Mathiasson, "Application of standard addition methodology for the determination of banned dyes in different leather types," Analytica Chimica Acta, 552: 76-80, 2005.

2. L. H. Ahlstrom, E. Bjorklund, L. Mathiasson, "Optimization of an analytical procedure for the determination of banned azo dyes in leather," Anal Bioanal Chem, 382: 1320-1327, 2005.

Analysis of herbicides in drinking water using the Agilent 1120 Compact LC

Application Note

Angelika Gratzfeld-Huesgen



Abstract

The Agilent 1120 Compact LC is the system of choice for conventional, analytical scale liquid chromatography. It is an intergrated LC designed for ease of use, performance and reliability. It is ideally suited for the analysis of herbicides on account of its capability to achieve highly precise retention times and peak areas, and low detection limits for the analyzed compounds. In this Application Note, data is presented that demonstrates:

- Excellent retention time precision of less than 0.1 % RSD
- Excellent peak area precision of less than 0.5 % RSD
- Limit of detection (LOD) less than 150 pg for all herbicides analyzed

5989-7455EN

Introduction

The analysis of herbicides in various environmental and biological matrices is highly important in the protection of nature and the environment. In most countries, regulations limit the concentration of herbicides that are allowed in drinking water and other foodstuffs. Several analytical techniques are used in the control these limits. For compounds that are thermally unstable, high performance liquid chromatography (HPLC) is the recommended analysis technique.

In this study, nine herbicides were analyzed and the precision of retention times and peak areas was measured. Further, the limit of detection (LOD) of the compounds was determined. Drinking water spiked with trace amounts of the nine herbicides was used as sample.

Experimental

Equipment

- Agilent 1120 Compact LC comprising gradient pump with integrated degasser, autosampler with vial tray, column oven and variable wavelength detector, see figure 1
- Agilent HC-C18(2), high carbon load, 150 x 4.6 mm, 5 µm particle size column
- Agilent EZChrom Elite Compact software
- Nine herbicides were selected for the experiments and purchased from Sigma/Aldrich, see table 1.

Chromatographic conditions

- Mobile phase: A: Water, B: ACN
- Gradient: 10 to 90 %B in 15 min
- Flow rate: 1.5 mL/min
- Injection volume:

Figure 1
Agilent 1120 Compact LC

  5 µL for 1:100 dilution
  20 µL for 1:1000 dilution
  20 µL for 1:10,000 dilution
- Column temperature: 40 °C
- Detection wavelength: 225 nm
  Peakwidth: > 0.0025 min
  Response time: 0.06 s

Results and discussion

Analyzing herbicides with UV detection means that precision of retention times is of utmost importance. In addition, precision of peak areas must be less than
1 % in the low ng range.
According to the United States Environmental Protection Agency the tolerance level for diuron in different food commodities is about 0.1–2.0 ppm. This corresponds to about 1–20 ng per 10 µL. For simazine, the tolerance level in different commodities is 0.02–0.25 ppm, corresponding to 200–2500 pg per 10 µL.

As a consequence the demands on this application can be summarized as follows.

- Relative standard deviation of retention times less than 0.1 %
- Relative standard deviation of peak areas (in low ng range) less than 1 %
- Limit of detection (LOD) less than 150 pg for a 20 µL injection

The 1:100 dilution of the stock solution was used to evaluate the chromatographic conditions and the resulting chromatogram is shown in figure 2.

Compound	Stock Solution mg/10 mL	1:100 dilution 5 µL inj. vol. (ng per inj.)	1:1000 dilution 20 µL inj. vol. (ng per inj.)	1:10,000 dilution 20 µL inj. vol. (ng per inj.)
Metamitron	3.3	16.5	6.6	0.66
Chloridazone	2.5	12.5	5	0.5
Simazine	2.5	12.5	5	0.5
Cyanazine	3.7	18.5	7.4	0.74
Prometryn	4.8	24	9.6	0.96
Chlortoluron	9.6	48	19.2	1.920
Diuron	2	10	4	0.4
Propazine	4.6	23	9.2	0.92
Terbuthylazine	5.8	29	11.6	1.160

Table 1
Analyzed Herbicides and used concentrations.

To evaluate the relative standard deviation six runs were performed using the 1:1000 dilution of the stock solution and the results are shown in table 2.

The limits of detection were determined using 20 µL injections of the 1:10,000 dilution of the stock solution. The results are shown in figure 3 and table 3.

Peak	Compound	% RSD Ret. Times	% RSD Areas
1	Metamitron	0.07	0.19
2	Chloridazone	0.07	0.17
3	Cyanazine	0.05	0.10
4	Simazine	0.05	0.12
5	Prometryn	0.06	0.39
6	Diuron	0.02	0.34
7	Propazine	0.02	0.16
8	Terbuthylazine	0.02	0.33
9	Chlortoluron	0-04	0.26

Table 2
Relative standard deviations of retention times and peak areas in the low ng range.

Peak	Compound	LOD with S/N = 3 P-to-P noise 0.02 mAU (pg)
1	Metamitron	132
2	Chloridazone	50
3	Cyanazine	49
4	Simazine	50
5	Prometryn	144
6	Diuron	34
7	Propazine	34
8	Terbuthylazine	37
9	Chlortoluron	128

Table 3
Limits of detection for the analyzed herbices.

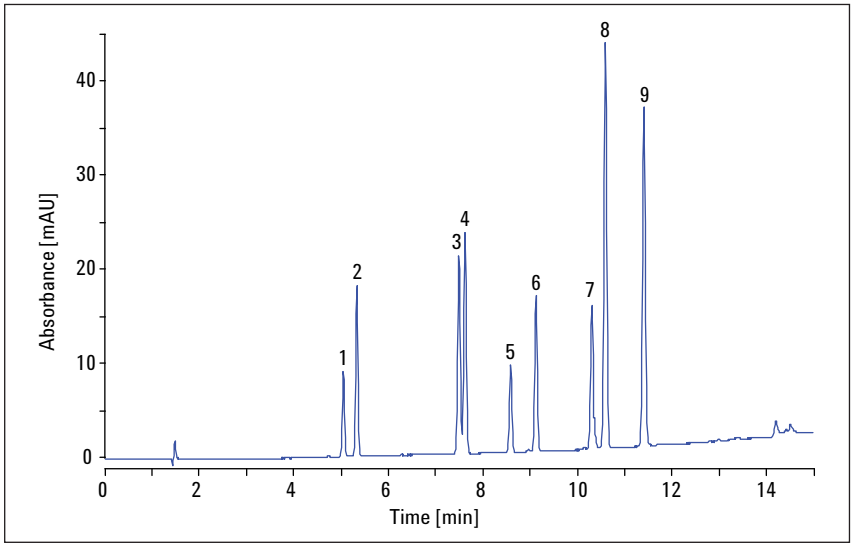


Figure 2
Chromatogram from analysis of the 1:100 dilution for evaluation of chromatographic conditions.

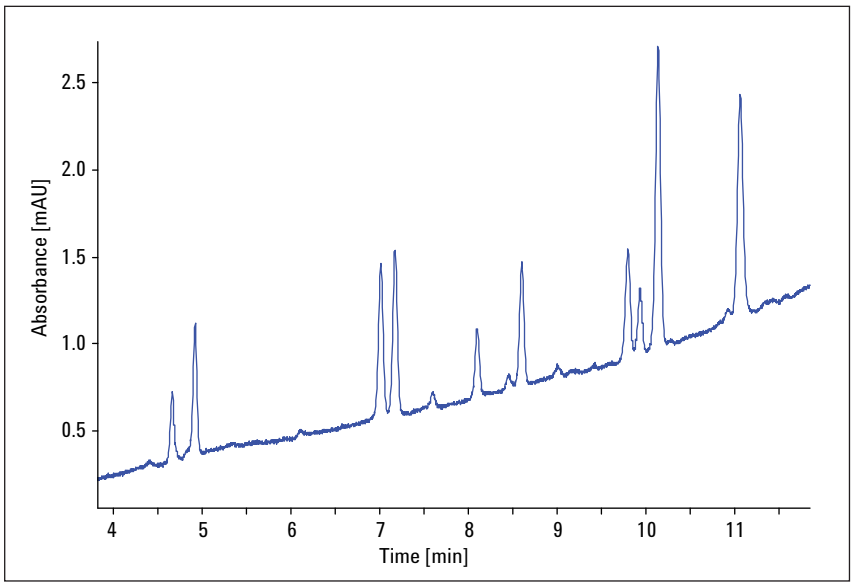


Figure 3
Chromatogram from analysis of the 1.10,000 dilution with an injection volume of 20 µL.

Analysis of herbicides in drinking water

600 µL of drinking water was spiked with 100 µL of the 1:1000 dilution and 300 µL of acetonitrile. Acetonitrile was added to avoid adhesion of the trace amounts of the herbicides on the glass surface of the vial. The resulting concentration was equivalent to the 1:10,000 dilution of the stock solution. Figure 4 shows the chromatograms of the analyses of the blank drinking water sample (lower trace), spiked drinking water sample (center trace) and the 1:10,000 dilution of the stock solution. The chromatographic conditions were the same as for the analysis shown in figure 1.

The chromatograms showed that these herbicides can be analyzed in the low pg range using the described chromatographic conditions.

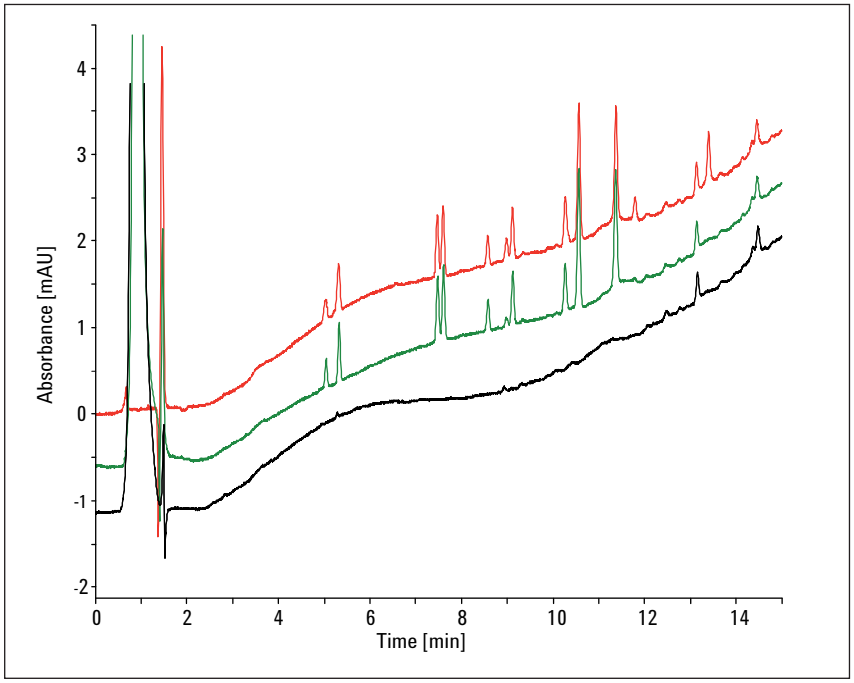


Figure 4
Chromatogram of analysis of spiked drinking water:
Upper trace – 1:10,000 diluted stock solution
Center trace – spiked drinking water sample
Lower trace – blank drinking water sample

Conclusion

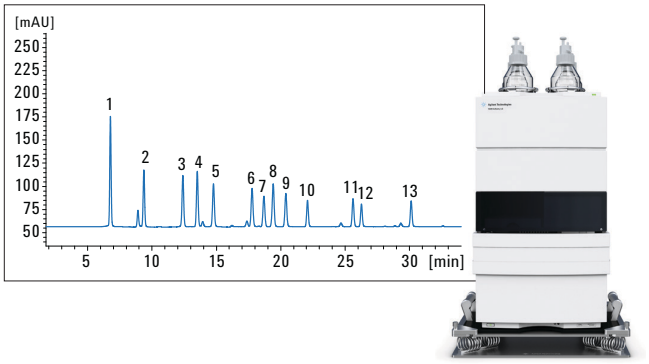
The Agilent 1120 Compact LC was used for the analysis of herbicides in drinking water. This instrument was able to analyze these compounds at levels as low as 0.02 ppm, for example, for simazine. The identification of these compounds using retention times was based on the excellent retention time precision of less than 0.08 %. The quantification in the low nanogram range yielded relative standard deviations less than 0.4 % for the peak areas and allowed accurate determination of the compounds.

Analysis of DNPH-derivatized Aldehydes and Ketones using the Agilent 1220 Infinity LC System with Diode Array Detector

Application Note

Author

Sonja Schneider
Agilent Technologies, Inc.
Waldbronn, Germany



Abstract

Aldehydes and ketones are important compounds in the chemical industry. However, these compounds can be hazardous when released into the environment. This Application Note describes the successful analysis of 13 DNPH-derivatized aldehydes and ketones using the Agilent 1220 Infinity Gradient LC system with built-in diode-array detector (DAD). In combination with the Agilent 1220 Infinity Mobile Upgrade Kit, the system can be installed in a vehicle and measurements can be performed at different test sites. Using both 1.8-µm and 5-µm columns achieved excellent precision and linearity as well as low limits of detection (LOD) and limits of quantification (LOQ) for all carbonyl-containing compounds.

Introduction

The environmental analysis of aldehydes and ketones is important due to the toxic and potentially carcinogenic nature of some of these carbonyl compounds. As combustion products from, for example, automotive exhaust or tobacco smoke, aldehydes and ketones are significant air pollutants and can also be detected in water and soil. Atmospheric carbonyl compounds are also created by thermal degradation of polymers in the plastic processing industry.

Of all the atmospheric carbonyl compounds, acetaldehyde and formaldehyde are the most abundant. In addition, formaldehyde has the largest technical relevance because it an essential precursor for polymers and many other chemical compounds. It is also frequently used as disinfectant and preservative (for example, formalin). Also, acetaldehyde is one of the most important aldehydes because it is highly prevalent in nature and a significant compound in the chemical industry. Many other aldehydes and ketones are also found in the chemical industry, for example, in rubber, synthetic resin, and plastic production.

The determination of aldehydes and ketones in several environmental matrices is mostly conducted with high pressure liquid chromatography (HPLC) with UV detection based on the derivatization of the carbonyl compounds with 2,4-dinitrophenylhydrazine (DNPH).

This Application Note presents the analysis of DNPH-derivatized aldehydes and ketones using the 1220 Infinity Gradient LC system with built-in DAD. As an integrated, binary-gradient liquid chromatography (LC) system with a pressure range of up to 600 bar, 5-µm and 1.8-µm columns could be deployed due to the ultra-HPLC (UHPLC) capabilities of the system. Using the 1220 Infinity LC Mobile Upgrade Kit, the following application can be carried out in a mobile laboratory.

Experimental

Instrumentation

The Agilent 1220 Infinity Gradient LC System (G4294B) was equipped with a dual-channel gradient pump with integrated degassing unit, autosampler, column compartment and diode array detector. For transportation, the system was mounted on a transportation plate, Agilent 1220 Infinity Mobile Upgrade Kit (G4292A).

Software

- Agilent OpenLAB CDS ChemStation Edition for LC & LC MS Systems, Rev. C.01.04 [35]
- Agilent OpenLAB CDS 3D UV Add-On Software.

Sample

The mixture of aldehyde-2,4-dinitrophenylhydrazones and ketone-2,4-dinitrophenylhydrazones was certified reference material from Sigma-Aldrich (Catalog No. 47651-U) diluted in acetonitrile. In the mixture, each analyte had a concentration of 30 µg/mL of carbon. For analysis, the mixture was diluted in dimethyl sulfoxide (DMSO) in a ratio of 1:10. Table 1 shows the elution order for all analytes depicted in all figures. Acetone and DMSO were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak).

Peak	Substance
1	Formaldehyde-2,4-dinitrophenylhydrazone
2	Acetaldehyde-2,4-dinitrophenylhydrazone
3	Acrolein-2,4-dinitrophenylhydrazone
4	Acetone-2,4-dinitrophenylhydrazone
5	Propionaldehyde-2,4-dinitrophenylhydrazone
6	Crotonaldehyde-2,4-dinitrophenylhydrazone
7	Methacrolein-2,4-dinitrophenylhydrazone
8	2-Butanone-2,4-dinitrophenylhydrazone
9	Butyraldehyde-2,4-dinitrophenylhydrazone
10	Benzaldehyde-2,4-dinitrophenylhydrazone
11	Valeraldehyde-2,4-dinitrophenylhydrazone
12	*m*-Tolualdehyde-2,4-dinitrophenylhydrazone
13	Hexaldehyde-2,4-dinitrophenylhydrazone

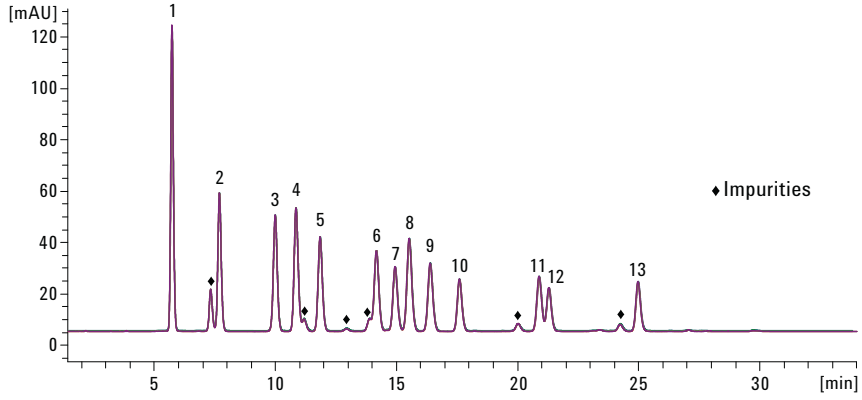
Table 1
Elution order of aldehyde and ketone mixture.

Chromatographic conditions

Parameter	Conditions (4.6 mm id columns)	Conditions (2.1 mm id columns)
Columns:	Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm (p/n 959993-902) Agilent ZORBAX RRHT Eclipse Plus C18, 4.6 × 150 mm, 1.8 µm (p/n 959994-902)	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm (p/n 959759-902)
Mobile Phase:	A: Water B: Acetone	A: Water B: Acetone
Flow rate:	1.2 mL/min	0.25 mL/min
Gradient:	0 minutes: 45 %B 12 minutes: 53 %B 28 minutes: 67 %B 32 minutes: 67 %B 33 minutes: 95 %B	0 minutes: 45 %B 12 minutes: 53 %B 28 minutes: 67 %B 32 minutes: 67 %B 33 minutes: 95 %B
Stop time:	34 minutes	34 minutes
Post time:	20 minutes	20 minutes
Injection volume:	10 µL	2.1 µL
Temperature:	45 °C	45 °C
Detection:	360 nm/10 nm Ref.: off Peak width > 0.025 minutes (0.5 second response time, 10 Hz)	360 nm/10 nm Ref.: off Peak width > 0.025 minutes (0.5 second response time, 10 Hz)

Results and Discussion

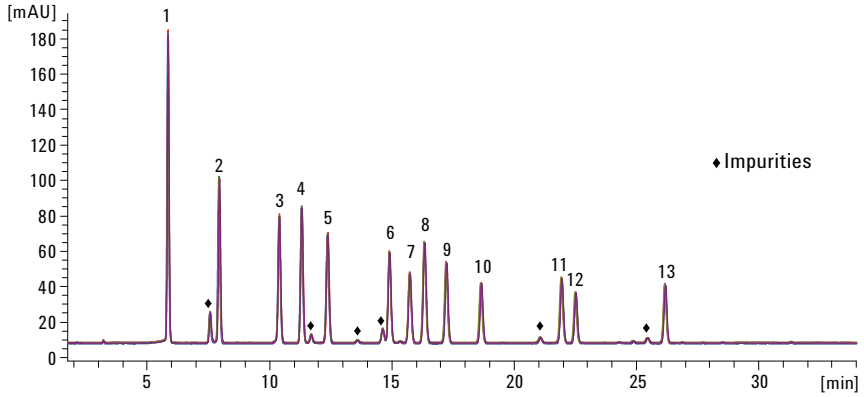
The DNPH-derivatized aldehydes and ketones were separated using an Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm column and the relative standard deviation (RSD) of retention times and areas was determined for six consecutive runs, see Figure 1. All RSDs of the retention times were below 0.01 %. The area precision was found to be below 0.4 %. In addition to the 13 carbonyl compounds, some impurities were detected.



Peak	RSD RT (%)	RSD Area (%)
1	0.060	0.230
2	0.051	0.198
3	0.051	0.239
4	0.048	0.219
5	0.050	0.212
6	0.044	0.221
7	0.049	0.168
8	0.050	0.172
9	0.049	0.232
10	0.045	0.268
11	0.044	0.326
12	0.040	0.288
13	0.034	0.239

Figure 1
Six consecutive runs of DNPH-derivatized aldehydes and ketones separated on an Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm column at 90 bar.

The same sample was also separated using a ZORBAX RRHT Eclipse Plus C18, 4.6 × 150 mm, 1.8 µm column at a higher pressure of 420 bar. The RSD of retention times and areas was determined for six consecutive runs, see Figure 2.



Peak	RSD RT (%)	RSD area (%)
1	0.115	0.526
2	0.150	0.163
3	0.078	0.318
4	0.082	0.340
5	0.080	0.341
6	0.054	0.289
7	0.060	0.350
8	0.063	0.313
9	0.077	0.283
10	0.084	0.187
11	0.065	0.248
12	0.071	0.394
13	0.058	0.233

Figure 2
Six consecutive runs of DNPH-derivatized aldehydes and ketones separated on an Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 1.8 µm column at 420 bar.

The precision of retention times and areas were found similar to those achieved with the 5-µm column. All RSDs of the retention times were below 0.016 %. The area precisions were found to be below 0.4 %. Using sub-two µm columns, an improvement in resolution was visible by a factor of approximately two, especially visible for peak 11 and 12, see Figure 2 and Table 2.

Peak	Resolution (5-µm columns)	Resolution (1.8-µm columns)
1	–	-
2	1.45	2.35
3	8.41	14.50
4	2.88	4.78
5	2.01	3.32
6	0.89	1.27
7	2.18	3.75
8	1.58	2.61
9	2.29	3.89
10	3.33	6.41
11	2.29	3.68
12	1.09	2.55
13	2.02	3.14

Table 2
Improvement in resolution from 5-µm to 1.8-µm columns.

Using the ZORBAX RRHT Eclipse Plus C18 4.6 × 150 mm, 1.8 µm column, the linearity, LOD and LOQ were determined for a dilution series of the DNPH-derivatized aldehydes and ketones from 3 µg/mL down to 4 ng/mL. An excellent correlation was found for all carbonyl-containing compounds. All correlation curves showed very high linearity with correlation factors of 1 or 0.99999, see Table 3.

Compound	Correlation coefficient
Formaldehyde-2,4-dinitrophenylhydrazone	1
Acetaldehyde-2,4-dinitrophenylhydrazone	0.99999
Acrolein-2,4-dinitrophenylhydrazone	1
Acetone-2,4-dinitrophenylhydrazone	1
Propionaldehyde-2,4-dinitrophenylhydrazone	1
Crotonaldehyde-2,4-dinitrophenylhydrazone	1
Methacrolein-2,4-dinitrophenylhydrazone	1
2-Butanone-2,4-dinitrophenylhydrazone	1
Butyraldehyde-2,4-dinitrophenylhydrazone	1
Benzaldehyde-2,4-dinitrophenylhydrazone	1
Valeraldehyde-2,4-dinitrophenylhydrazone	1
m-Tolualdehyde-2,4-dinitrophenylhydrazone	0.99999
Hexaldehyde-2,4-dinitrophenylhydrazone	1

Table 3
Correlation factors of all carbonyl components.

Figure 3 shows the correlation curves of four of the compounds together with the response factors, which were in the ± 5 % deviation range.

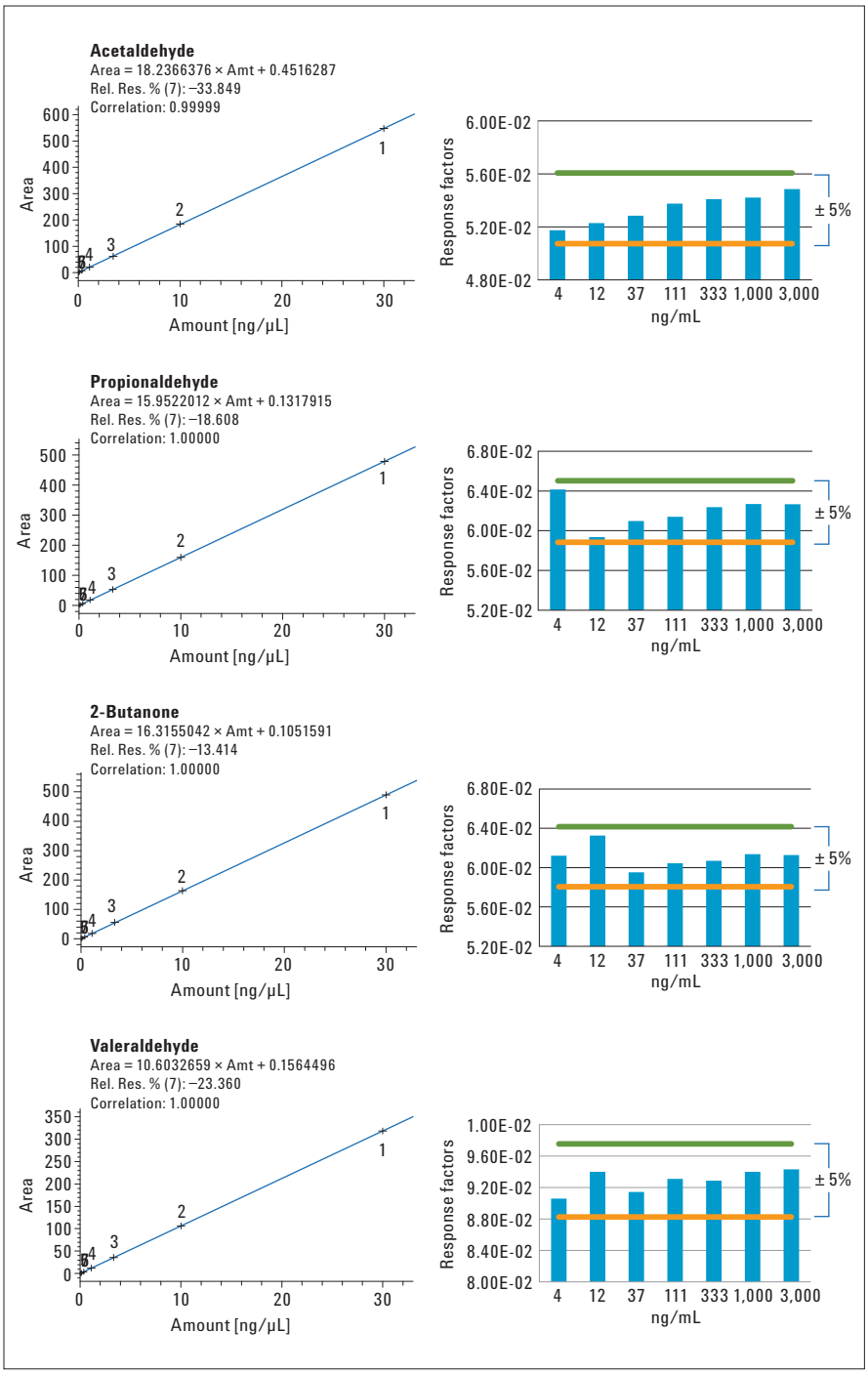


Figure 3
Linearity of four of the carbonyl compounds.

The LOD and LOQ were evaluated from the concentration of aldehydes and ketones required to give a signal-to-noise ratio of at least 3 and 10, respectively. Table 4 displays the LOD and LOQ for all the components.

Figure 4 shows the separation of the carbonyl compounds on a narrow bore ZORBAX RRHD Eclipse Plus C18, 2.1 x 150 mm, 1.8 µm column. With this setup, it was possible to save approximately 80 % of sample and mobile phase due to the adjustment of injection volume and flow rate to 2.1 id columns.

Conclusions

DNPH-derivatized aldehydes and ketones were successfully analyzed using the Agilent 1220 Infinity Gradient LC with DAD using 5-µm as well as 1.8-µm columns under UHPLC conditions. Excellent values for precision and linearity were achieved together with low LODs and LOQs for all carbonyl components. Although the 1220 Infinity Gradient LC is designed for 4.6 id columns, applications using 2.1 id columns are possible under nonfast gradient conditions.

Compound	LOD [pg]	LOQ [pg]
Formaldehyde-2,4-dinitrophenylhydrazone	8	26
Acetaldehyde-2,4-dinitrophenylhydrazone	15	51
Acrolein-2,4-dinitrophenylhydrazone	19	63
Acetone-2,4-dinitrophenylhydrazone	18	61
Propionaldehyde-2,4-dinitrophenylhydrazone	24	79
Crotonaldehyde-2,4-dinitrophenylhydrazone	29	96
Methacrolein-2,4-dinitrophenylhydrazone	37	124
2-Butanone-2,4-dinitrophenylhydrazone	25	85
Butyraldehyde-2,4-dinitrophenylhydrazone	34	112
Benzaldehyde-2,4-dinitrophenylhydrazone	41	138
Valeraldehyde-2,4-dinitrophenylhydrazone	38	127
m-Tolualdehyde-2,4-dinitrophenylhydrazone	47	156
Hexaldehyde-2,4-dinitrophenylhydrazone	43	145

Table 4
LOD and LOQ for all carbonyl components.

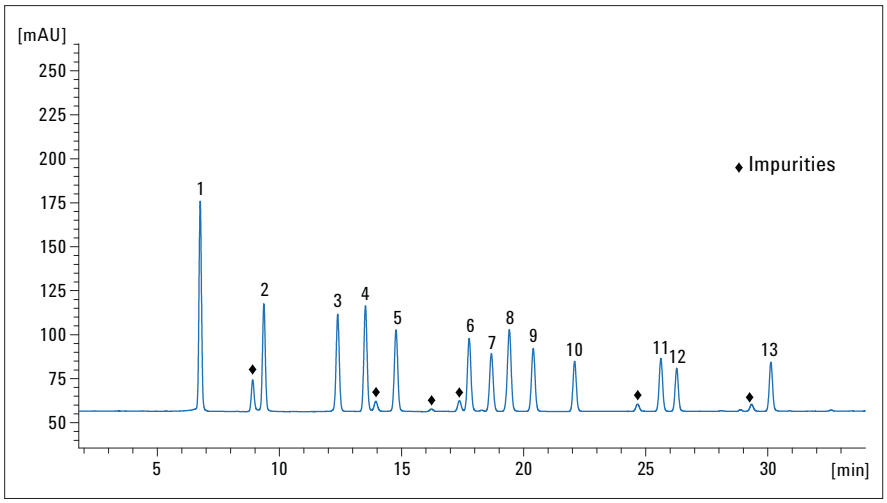


Figure 4
DNPH-derivatized aldehydes and ketones using an Agilent ZORBAX Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm.

Food Applications

Food and beverages need to be provided of consistent quality and uncompromising safety. Especially quality control of incoming ingredients (raw material) or the final product are part of this application area, for example, perservatives and melamine testing.

Analysis of plant stanyl fatty acid esters in enriched margarine using an on-line coupled Agilent 1220 Infinity LC-7890 GC System

Application Note

Food

Authors

Andreas Barnsteiner, Rebecca Esche, Karl-Heinz Engel
Technische Universität München
Lehrstuhl für Allgemeine Lebensmitteltechnologie
Maximus-von-Imhof-Forum 2
85350 Freising, Germany

Walter Kohlert, Stefan Fenzel
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

This Application Note describes the investigation of intact plant stanyl fatty acid esters in an enriched commercial margarine using an on-line coupled Agilent 1220 Infinity LC-7890A GC system. The lipid extract was directly analyzed without prior purification steps. The LC fraction of plant stanyl esters was transferred on-line into the GC system using the solvent vent mode of the multimode inlet for solvent evaporation. The on-line LC-GC combination showed very good linearity and repeatability.

Introduction

Plant steryl and stanyl esters (Figure. 1) are added to food products like skimmed milk-drinking yogurts or margarines because of their cholesterol-lowering properties. The capillary gas chromatographic investigation of plant stanyl fatty acid esters from skimmed milk products can be performed directly after the lipid extraction[1]. However, the presence of di- and triglycerides may hamper the direct GC quantification. Therefore, the analysis in lipid extracts from foods with high fat contents like margarine requires a fractionation prior to the GC separation by laborious off-line techniques, such as TLC or SPE. The on-line coupling of LC and GC offers an efficient and elegant alternative. The plant stanyl esters can be fractionated by liquid chromatography and transferred on-line into the GC system. In this way, the pre-fractionation step and the capillary gas chromatographic analysis of the transferred LC fraction are performed in a closed system in one run. Hence, the risk of sample loss and contamination is reduced and the approach results in better repeatability[2, 3].

In a recently published paper[1], the analysis of plant stanyl esters in enriched margarines using an on-line LC-GC system equipped with a loop-type interface was reported. Using the loop-type interface, the solvent evaporation was performed in the GC capillary columns by means of a pre-column system in combination with an early solvent vapor exit. Due to the high solvent amounts which were loaded on the pre-column system with each transfer, a loss of resolution was observed after a few runs.

The on-line coupling of an Agilent 1220 Infinity LC system and an Agilent 7890A GC system, with a 2-position/6-port switching valve using the solvent vent mode of the multimode inlet of the GC, allowed for the evaporation of the solvent prior to the capillary column[4]. A pre-column system and/or a solvent vapor exit were not necessary. This combination was already suitable for the analysis of cholesteryl esters[4].

In this Application Note, the use of an Agilent on-line coupled LC-GC combination for the quantification of plant stanyl esters in enriched margarine is presented.

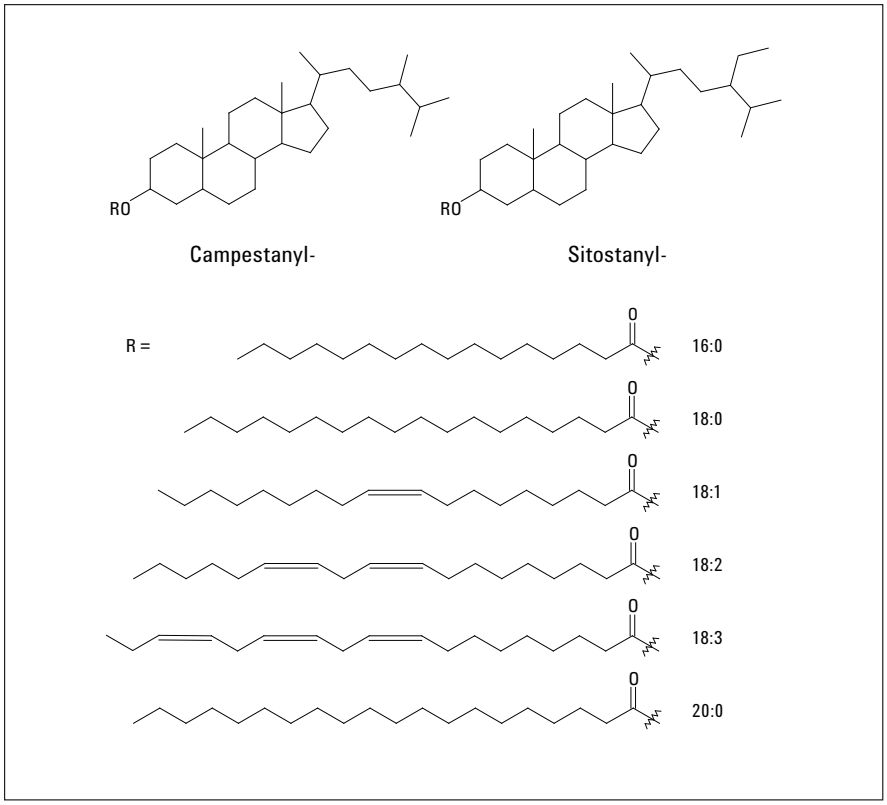


Figure 1
Structures of plant stanyl fatty acid esters.

Experimental

Chemicals and materials

The plant stanyl ester mixture "plant stanol ester, STAEST-115" was provided by Raisio Group (Raisio, Finland). The internal standard cholesteryl palmitate (≥ 98%) was obtained from Sigma Aldrich (Taufkirchen, Germany). Benecol (taste-type Kevyt kasvirasvalevite 32%, with added plant stanyl esters) margarine was purchased in a supermarket in Finland; the plant stanol content was labeled as 8 wt-%, total lipids as 32 wt-%.

Sample preparation of margarine[1]

The margarine sample (20–40 mg, accuracy of ± 0.1 mg) was weighed into a vessel; internal standard (cholesteryl palmitate, 750 µg), 5 mL of *n*-hexane/MTBE (3:2) and sodium sulfate (anhydrous) were added and sonicated for 1 minute. The solution was filtered through a 0.45 µm membrane filter assembled with a 5 mL syringe. The vessel and the filter were washed twice with 5 mL *n*-hexane/MTBE (3:2). After dilution (1:5) of the combined extracts, the solution was used for on-line LC-GC analysis.

Quantification

The five-point calibration functions of nine individual stanyl esters were generated in a range of 0.2 – 1.0 µg of total stanyl ester ("plant stanol ester, STAEST-115") per 2 µL i.v. Each calibration point was done in triplicate. Linear regression analysis was performed in coordinate ratios of areas (individual stanyl ester/IS) and amounts (individual stanyl ester/IS).

Equipment

The coupling of the Agilent 1220 Infinity LC system to the Agilent 7890A GC system was accomplished using an Agilent 2-position/6-port switching valve equipped with a 200 µL sample loop (Table 1). The evaporation of the eluent was performed using the temperature programmable MM Inlet in the PTV solvent vent mode[4].

Results and discussion

The chromatograms obtained by on-line LC-GC analysis of a margarine enriched with plant stanyl esters are presented in Figure 2. The LC-fractionation (Figure 2a) was performed isocratically on a silica gel column with *n*-hexane/MTBE (96+4; v+v) as mobile phase. The plant stanyl esters eluted after approximately 4 minutes. The transfer was performed 4.25 minutes after injection. The transfer conditions for the analysis of cholesteryl esters[4] were also suitable for plant stanyl esters. The GC separation of the transferred fraction was similar to that reported for the on-line LC-GC analysis via a loop type interface[1]. The intact plant stanyl fatty acid esters were distinguishable according to their carbon number and, in the case of unsaturated fatty acid moieties, to the number of double bonds; only the esters of saturated and monounsaturated fatty acids of the same chain length eluted at the same time.

Chromatographic conditions	
LC conditions	
Injection volume:	2 µL
Eluent:	*n*-hexane/*tert*-butylmethyl ether (96:4, v/v)
Column temperature;	27 °C
Column flow:	0.200 mL/min
Column type:	Eurospher-100Si (250 x 2 mm I.D., 5 µm)
Wavelength:	205 nm
LC controlled interface	
Transfer valve:	4.25 min: Position 1 → 2 7.50 min: Position 2 → 1
GC start:	4.20 min: Change contacts switch contact A to closed 4.25 min: Change contacts switch contact A to open
GC conditions	
Front MM inlet:	Mode: Solvent vent
	Carrier: H_2
	Pressure: 7.8 psi
	Septum purge flow: 3 mL/min
	Vent pressure: 4 psi until 0.5 min
	Vent flow: 1000 mL/min
	Temperature program: Initial: 50 °C for 0.5 min
	Rate 1: 900 °C/min to 350 °C for 2 min
	Purge flow to split vent: 2.5 mL/min at 0.5 min
	Gas saver: 20 mL/min after 5 min
Column number 1:	Column type: Restek Rtx-200MS: 30 m × 250 µm; 0.1 µm df;
	Constant flow: 1.5 mL/min
Column number 2:	Transfer line, controlled by PCM C-1
	Pressure program: Initial: 5 psi for 0.3 min
	Rate 1: 10 psi/min to 20 psi
Oven:	Temperature program: Initial: 40 °C for 2 min
	Rate 1: 100 °C/min to 100 °C for 0 min
	Rate 2: 15 °C/min to 310 °C for 2 min
	Rate 3: 1.5 °C/min to 340 °C for 3 min
Detector:	FID: 360 °C
	(H_2: 30 mL/min, Air: 400 mL/min; Makeup: 25 mL/min)

Table 1
Liquid and gas chromatographic conditions.

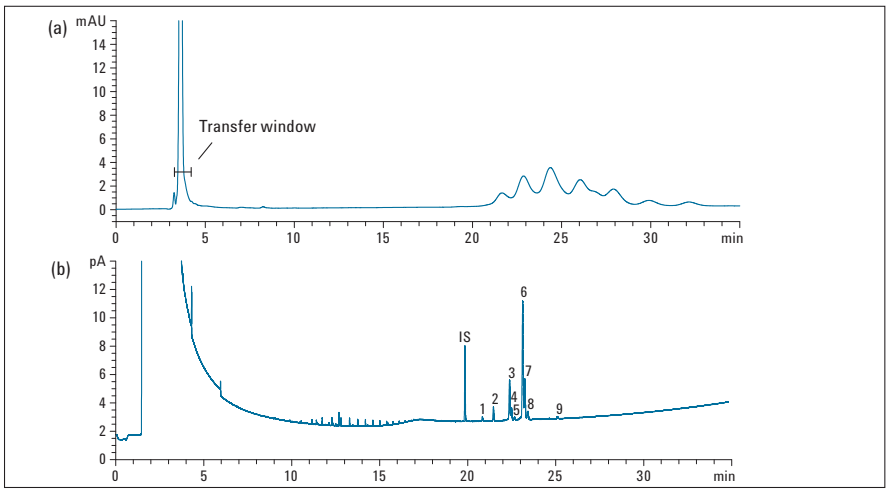


Figure 2
Analysis of plant stanyl esters in enriched margarine by online LC-GC/FID; (a) LC-chromatogram and (b) GC-chromatogram of the transferred LC-fraction; peak numbering according to Table 2; (IS) internal standard cholesteryl palmitate.

Under the employed on-line LC-GC conditions, using the Agilent Multimode Inlet for the solvent evaporation[4], the solvent load on the GC capillaries was low in comparison to the loop type coupled system. Even after 600 transfers, no loss of resolution was observed using the on-line LC-GC combination.

For the calibration, linear regression analysis was performed in the coordinate ratios of areas (individual stanyl ester/IS) and amounts (individual stanyl ester/IS). The correlation coefficients of the calculated calibration functions (R^2) were in the range of 0.995 – 0.999, showing very good linearity of the on-line LC-GC/FID detector response.

The repeatability was determined by 10-fold injections of the same sample solution. The coefficients of variation were low (< 9%) for all plant stanyl esters (Table 2). The quantitative results were comparable to those obtained by means of the loop type interface coupled on-line LC-GC[1].

		CV [%][b]			
No.[a]	**Stanyl ester**	**Extract 1**	**Extract 2**	**Extract 3**	**Amount [g/100 g][c]**
1	Campestanyl-16:0/16:1	7.7	2.0	5.1	0.18 ± 0.01 (8.1)
2	Sitostanyl-16:0/16:1	5.7	0.7	2.6	0.47 ± 0.02 (5.3)
3	Campestanyl-18:0/18:1	1.1	0.8	0.6	2.10 ± 0.06 (2.8)
4	Campestanyl-18:2	3.7	1.6	3.2	0.73 ± 0.04 (5.2)
5	Campestanyl-18:3	8.8	2.8	6.9	0.30 ± 0.02 (7.6)
6	Sitostanyl-18:0/18:1	1.1	0.9	1.5	6.36 ± 0.22 (3.5)
7	Sitostanyl-18:2	1.9	1.5	2.0	2.17 ± 0.11 (5.1)
8	Sitostanyl-18:3	6.5	2.2	7.2	0.81 ± 0.06 (8.0)
9	Sitostanyl-20:0/20:1	4.8	2.5	5.5	0.19 ± 0.01 (7.0)
Total stanyl esters		1.6	0.7	1.6	13.3 ± 0.5 (3.7)
Esterified sterols		1.6	0.7	1.6	8.1 ± 0.3 (3.7)

[a] Peak number correspond to Figure 2b

[b] Coefficient of variation [CV] determined by 10-fold injections of the same sample solution

[c] Values represent average ± standard deviations of 30 analyses (coefficient of variation [%])

Table 2
Coefficients of variation of plant stanyl esters.

Conclusion

On-line coupling of an Agilent 1220 Infinity LC system and an Agilent 7890A GC system was shown to be suitable for the quantitative analysis of plant stanyl fatty acid esters in enriched margarine. The on-line LC-GC system was characterized by easy handling and a very robust separation performance for both dimensions. Therefore, the Agilent on-line LC-GC combination can be a valuable tool for the routine analysis of plant steryl and stanyl esters in functional foods.

References

1.
A. Barnsteiner, T. Lubinus, A. di Gianvito, W. Schmid, K.-H. Engel, GC-Based Analysis of Plant Stanyl Fatty Acid Esters in Enriched Foods. Journal of Agricultural and Food Chemistry, 2011, 59, (10), 5204-5214.

2.
P. Dugo, G. Dugo, L. Mondello, On-line coupled LC-GC: theory and applications. LC-GC Eur. 2003, 16, (12a), 35-43.

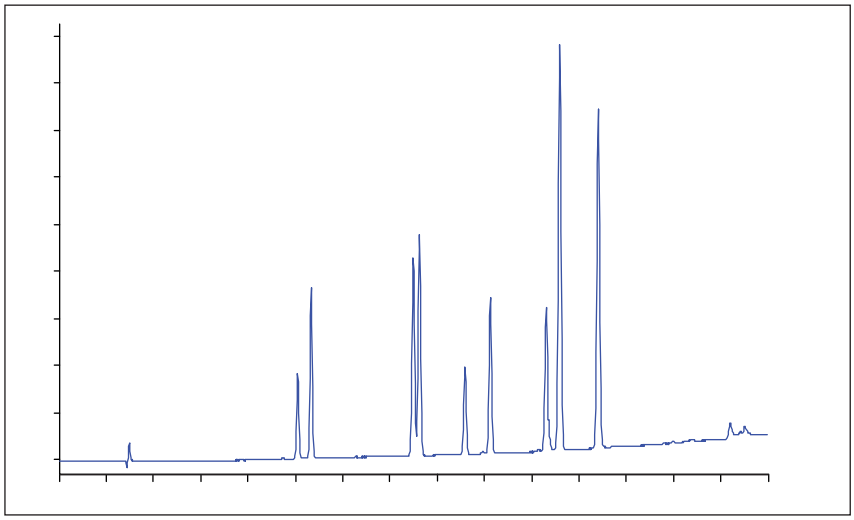
3.
T. Hyoetylaeinen, M.-L. Riekkola, On-line coupled liquid chromatography-gas chromatography. J Chromatogr A 2003, 1000, (1-2), 357-84.

4.
R. Esche, A. Barnsteiner, K.-H. Engel, W. Kohlert, S. Fenzel, "Two-dimensional chromatography: LC-GC online coupling of on Agilent 1260 Infinity LC and an Agilent 7890A GC", Agilent Technical Overview, publication number 5990-8025EN; **2011**

Analysis of antioxidants in chewing gum using the Agilent 1120 Compact LC

Application Note

Angelika Gratzfeld-Huesgen



Abstract

The Agilent 1120 Compact LC is the system of choice for conventional, analytical scale liquid chromatography. It is an intergrated LC designed for ease of use, performance and reliability. It is ideally suited for the analysis of antioxidants on account of its capability to achieve highly precise retention times and peak areas, and low detection limits for the analyzed compounds. The data in this Application Note demonstrates:

- Excellent retention time precision of less than 0.07 % RSD
- Excellent peak area precision of less than 0.3 % RSD for baseline separated peaks
- Limit of detection <1 to 75 ng for all antioxidants analyzed

5989-7456EN

Introduction

Antioxidants are widely used in the food industry. For example, butylated hydroxyanisole (BHA) is used in biscuits, fruit cake, candy and walnuts as well as in chewing gum. Estimated and proven risks of these compounds have led to regulations for the maximum allowed concentrations in foodstuffs. Some antioxidants such as BHA are not allowed in baby foods because of their influence on the accelerated digestion of vitamin D. BHA also has a detrimental influence on the blood level of lipids and cholesterol.

In this study, eight antioxidants were analyzed and the precision of retention times and areas was measured. Further, the limit of detection (LOD) of the compounds was determined. As a real-life example, the level of BHA in chewing gum was measured.

Experimental

Equipment

- Agilent 1120 Compact LC comprising gradient pump with integrated degasser, autosampler with vial tray, column oven and variable wavelength detector, see figure 1
- Agilent HC-C18(2), high carbon load, 150 x 4.6 mm, 5 µm particle size column
- Agilent EZChrom Elite Compact software

Chromatographic conditions

- Mobile phase:
  A: Water + 0.045 % TFA
  B: ACN + 0.045 % TFA
- Gradient: 10 to 90 %B in 15 min
- Flow rate: 1.5 mL/min
- Injection volume: 5 µL
- Column temperature: 40 °C
- Detection wavelength: 260 nm
  Peakwidth: > 0.0025 min
  Response time: 0.06 s

Sample

1. Vitamin C
2. Propyl gallat (PG)
3. 2,4,5-trihydroxy-butyrophenone (THBP)
4. mono-tert-butyl-hydroquinone (TBHQ)
5. Butylated hydroxyanisole (BHA)
6. 4-hydroxymethyl-2.6-di(tert-butyl)phenol (ionox 100)
7. Butylated-hydroxytoluene (BHT)
8. Ascorbyl-palmitate (ACP)

Results and discussion

Eight antioxidants were chosen for the determination of precision of retention time and areas. The limits of detection were also evaluated. All compounds were separated with excellent resolution, using conventional chromatographic conditions with a flow rate of 1.5 mL/min and a 15 min gradient, see figure 2.

Figure 1
Agilent 1120 Compact LC

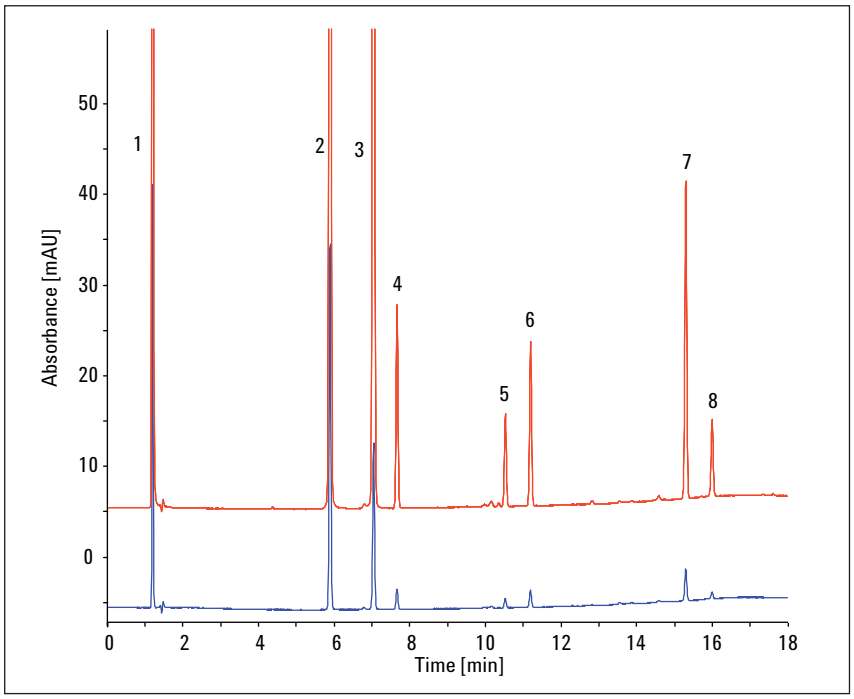


Figure 2
Upper trace–Antioxidant standards, 1350-200ng, 1:10 dilution
Lower trace–Antioxidant standards, 135-20ng, 1:100 dilution

The concentration levels used in this study are listed in table 1. The precision of retention times and areas was determined using the 1:10 dilution. The results are shown in table 2.

Even for peaks with heights less than or equal to 20 mAU, the precision of areas was less than 0.3 %, which is an excellent result. The area precision for vitamin C (peak 1) was affected by the continuous decomposition of this compound in solution. The precision of ACP (peak 8) was influenced by a small broad peak that eluted just before the ACP peak. Both peaks were not completely separated and quantification at about 12 mAU was not as good as for the other fully separated compounds.

The precision of retention times for all compounds was less than 0.05 % relative standard deviation (RSD). Figure 2 shows the overlaid chromatograms six consecutive runs.

The limit of detection was calculated based on the chromatogram of the 1:100 dilution, see figure 1. The results are shown in table 3.

Peak	Compound	LOD with S/N = 3 1:100 dilution (ng)
1	Vitamin C	<1*
2	PG	<1
3	THBP	75
4	TBHQH	6
5	BHA	3
6	Ionox100	2.3
7	BHT	2.5
8	ACP	2

Table 3
Limits of detection for the antioxidants (*rapid decomposition made determination of traces difficult).

Peak	Compound	Stock Solution mg/10 mL	1:10 dilution 5 µL inj. vol. (ng per inj.)	1:100 dilution 5 µL inj. vol. (ng per inj.)
1	Vitamin C	20	1000	100
2	PG	21	1050	105
3	THBP	15	750	75
4	TBHQH	21	1050	105
5	BHA	11	500	50
6	Ionox100	14	700	70
7	BHT	27	1350	135
8	ACP	4	200	20

Table 1
Concentration levels of the analyzed antioxidants.

Peak	Compound	% RSD Ret. Times	% RSD Areas	Peak height (mAU)
1	Vitamin C	0.04	2.01*	~200
2	PG	0.03	0.08	~400
3	THBP	0.02	0.12	~200
4	TBHQH	0.01	0.23	<25
5	BHA	0.02	0.21	<15
6	Ionox 100	0.01	0.10	<20
7	BHT	0.01	0.26	<50
8	ACP	0.01	1.74**	~12

Table 2
Precision of retention times and areas (*decomposition; **integration problem with front peak).

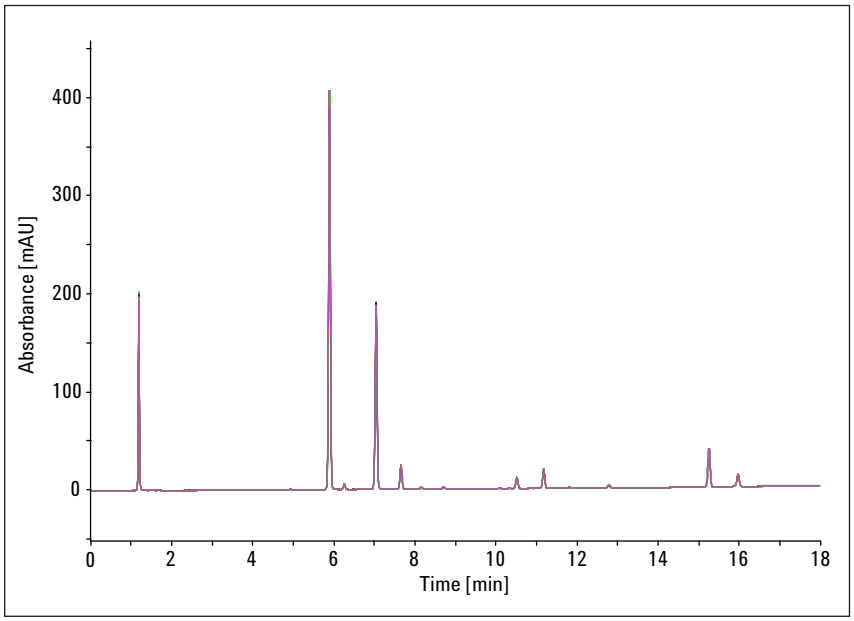


Figure 3
Overlay of six consecutive runs.

The BHA content in chewing gum was determined using the method developed in this study. Figure 4 shows complete chromatograms of standard and chewing gum extract. Figure 5 shows the part of the chromatogram between 9 and 13 minutes enlarged for more detailed. The sample was prepared by cutting 14.1 g of sugar-free chewing gum in small pieces and extracting with acetonitrile in an ultrasonic bath for 30 min. See figure 1 for other conditions. About 25 ppb BHA could be determined in 1 g of chewing gum. This is a relative value because recovery rates were not evaluated.

Conclusion

The Agilent 1120 Compact LC was used for the analysis of antioxidants. This instrument was able to analyze these compounds with high precision. Retention time precision was less than 0.05 % RSD and area precicion of baseline separated peaks was less than 0.3 % RSD. The limit of detection was between 1 and 75 ng. In a real-life example, 25 ppb of BHA were found in 1 g of chewing gum after extraction with acetonitrile in an ultrasonic bath.

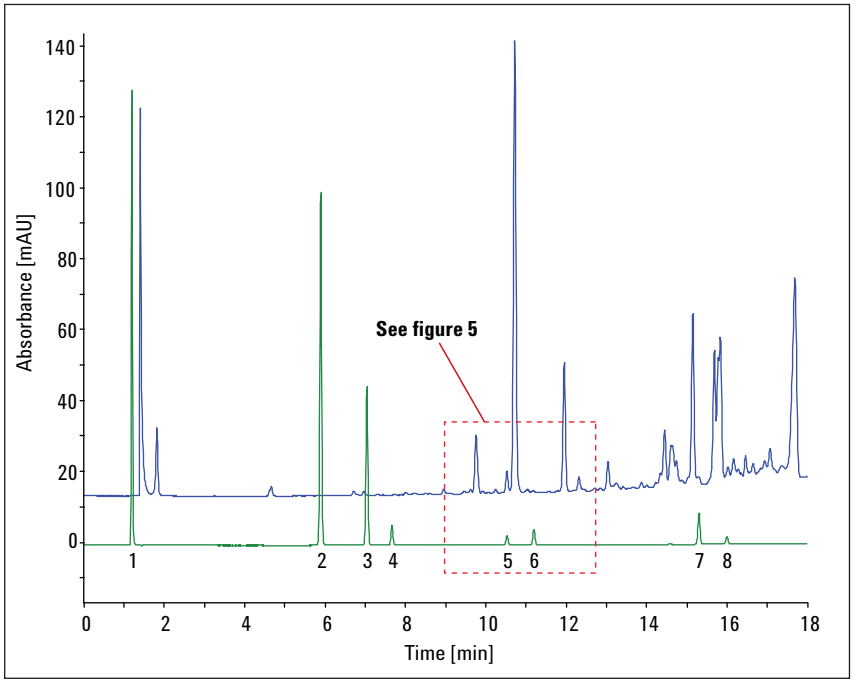


Figure 4
Upper trace–chewing gum extract, lower trace–antioxidant standards.

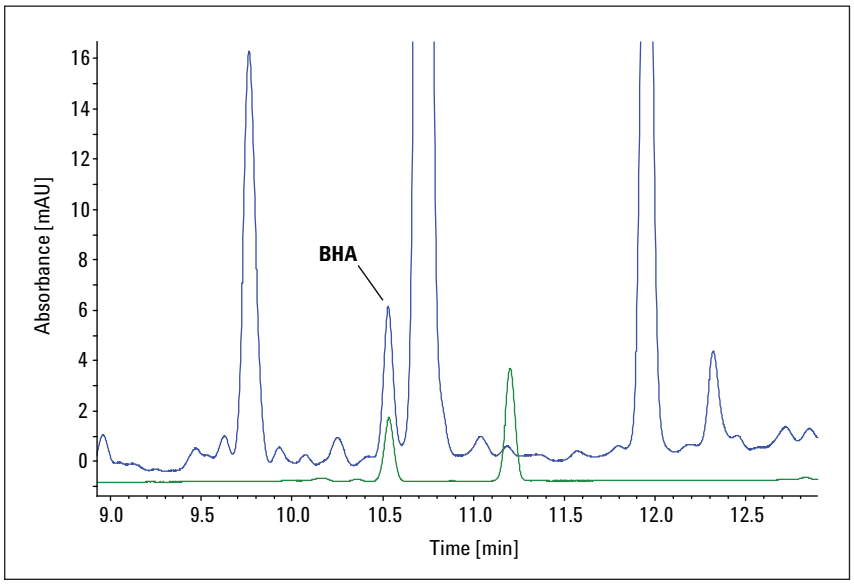
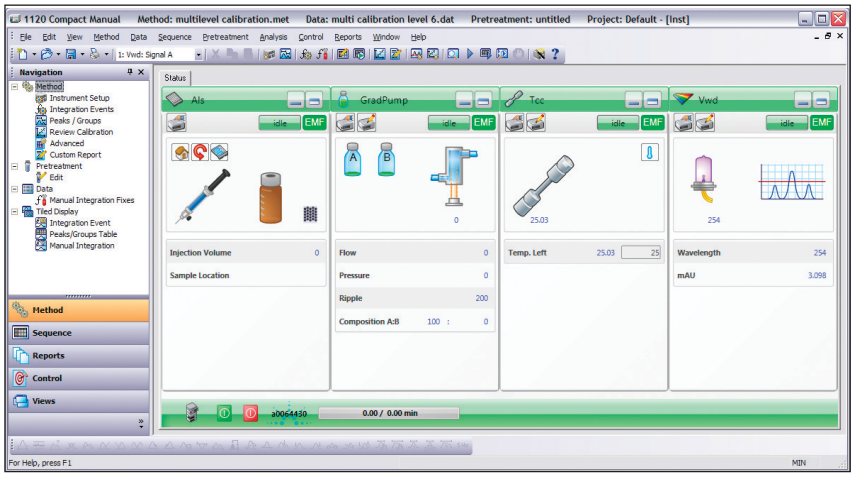


Figure 5
Enlarged view of figure 4, showing the chewing gum extract (upper trace) and the BHA peak at 10.55 min, equivalent to about 25 ng/g or 25 ppb.

Analysis of preservatives in food and cosmetics with the Agilent 1120 Compact LC system

Application Note

Rongjie Fu,
Zhixiu Xu



Abstract

An HPLC method was developed for simultaneous determination of the nine preservatives most often used in food and cosmetics. The system suitability results showed that the Agilent 1120 Compact LC is the system of choice for conventional, analytical scale liquid chromatography. This integrated HPLC system was designed for ease of use, performance, and reliability. The quantitative analysis of typical samples is demonstrated in this Application Note.

5989-8960EN

Introduction

Preservatives are very popular in the food and cosmetics industries because they prevent these products from degrading within the warranty time. But preservatives are strictly regulated because their overuse can cause some health problems in humans. For example, some preservatives can accumulate in the human body and negatively influence the metabolism process. Today's trends in food and cosmetics increasingly emphasize the concepts of healthy and green. That means use of safer raw materials, as well as fewer preservatives and control of preservatives within a safe limit.

In developing countries like China, the regulation of preservatives in food and cosmetics is approaching the international standards, such as the commonly used regulations adopted by the Codex Alimentarius Commission (CAC). In general, the regulations set the concentration limits on the preservatives in cosmetics and food. With increased research on safety of food and cosmetics, it might be necessary to analyze more preservatives in the future.

Some popular preservatives were analyzed in this application. A face conditioner and glace fruit were selected as typical samples that contain certain kinds of preservatives. The manufacturers of these products need to control the quality of their products before they go to market. The regulatory agencies check these products in the market very carefully to see if the amount of preservative is within the limit.

The experiments in this Application Note were performed with the Agilent 1120 Compact LC, which is the system of choice for conventional, analytical scale liquid chromatography. It is an integrated HPLC system designed for ease of use, performance, and reliability. It is ideally suited for routine analyses in the food and fine chemical industries because of its capability to achieve very precise retention times and peak areas, as well as low detection limits for the analyzed compounds.

Experimental

Equipment

- Agilent 1120 Compact LC system with gradient pump (degasser inside), autosampler, column compartment, and variable wavelength detector (VWD)
- EZChrom Elite Compact software

Chemicals and reagents

- Reference standards were purchased from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China.
- Water was obtained from a Millipore water purifier.
- Acetonitrile (Fisher Scientific) was HPLC purity. All other reagents were analytical purity.

Sample preparation

Glace fruit: The fruit was cut into pieces and 1.5 g was weighed and diluted to 10 mL with water. The mixture was treated with an ultrasonic for 15 minutes, and was filtered with a 0.45 µm filter prior to injection.
Face conditioner: 1 mL was diluted to 10 mL with water, and the sample was filtered with a 0.45 µm filter prior to injection.

Chromatographic conditions

- Column: Agilent HC-C18(2), 4.6 x 250 mm, 5 µm
- Mobile phase: A = 20 mM acetate buffer, pH 4.2; B = acetonitrile
- Gradient: 0 – 25 min, 30 %B – 45 %B
- Flow rate: 1 mL/min
- Wavelength:

0 min	260 nm
5 min	230 nm
5.6 min	260 nm
9.2 min	230 nm
10.2 min	260 nm

- Injection volume: 5 µL
- Temperature: 30 °C

Results and discussion

Development of a method for these preservatives in food and cosmetics must consider the run time and the separation of the nine compounds. The other fact that must be considered is that the matrix of the real samples may influence the separation. The HPLC system needs a column with good efficiency, and for quantitative analysis of the preservatives, it must deliver good precision for retention times and peak areas.

Because the compounds used as preservatives have different ultraviolet (UV) spectra, the wavelength program of the variable wavelength detector was used in this study to detect all the compounds at their most sensitive wavelength.

The overlaid chromatograms of two real samples and the preservative standards are shown in figure 1. The corresponding peak names are listed in table 1. In glace fruit, the benzoic acid and sorbic acid were found. In the face conditioner sample, only methylparaben was found.

The system reproducibility was tested with the nine compounds (table 1). The high precision of the retention times gives high confidence when comparing the standards and real samples.

The linear range of the standards was tested with this Agilent 1120 Compact LC system. The results are listed in table 2. The data shows that very good regression factors (values of r^2) were achieved for each compound.

The quantitative results from the two samples are shown in table 3.

Conclusion

The Agilent 1120 Compact LC is ideal for the routine analysis of preservatives in food and cosmetics. Excellent resolution and good separation were achieved, and system suitability experiments showed the robustness and high precision for this kind of application. The high precision of the retention times and peak areas ensures reliable results when quantitation is needed for quality control. The variable wavelength detector can be used with programmed wavelength to adjust to the maximum absorbance for all the compounds.

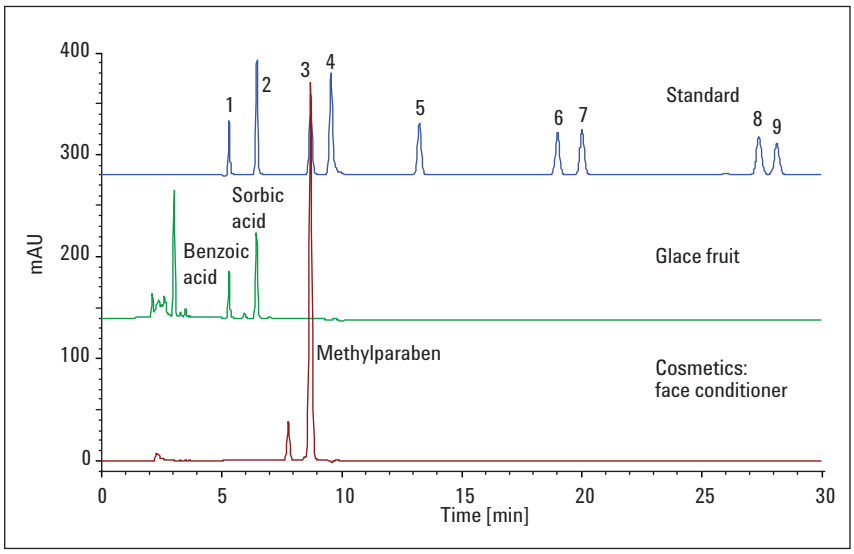


Figure 1
Overlaid chromatograms of preservative standards and real samples.

Peak	Compound	% RSD retention times	% RSD areas
1	Benzoic acid	0.03	1.11
2	Sorbic acid	0.03	0.16
3	Methylparaben	0.03	0.11
4	Dehydroacetic acid (DHA)	0.04	1.05
5	Ethylparaben	0.03	0.10
6	Isopropylparaben	0.03	0.11
7	n-Propylparaben	0.02	0.07
8	Isobutylparaben	0.02	0.10
9	n-Butylparaben	0.02	0.11

Table 1
Reproducibility of six injections of nine preservative standards.

Peak	Compound	Range (ng)	r^2
1	Benzoic acid	45.5 - 455	0.9998
2	Sorbic acid	35 - 350	0.9998
3	Methylparaben	66.5 - 665	0.9998
4	Dehydroacetic acid (DHA)	135 - 1350	0.9991
5	Ethylparaben	64.5 - 645	0.9998
6	Isopropylparaben	67 - 670	0.9998
7	n-Propylparaben	71.5 - 715	0.9998
8	Isobutylparaben	76.5 - 765	0.9998
9	n-Butylparaben	63.5 - 635	0.9998

Table 2
Linearity of the nine preservative standards.

	Benzoic acid	Sorbic acid	Methylparaben
Glace fruit	101.63 mg/Kg	70.59 mg/kg	ND*
Face conditioner	ND	ND	1.07 mg/mL

*ND = not detected

Table 3
The amount of preservatives in the real samples.

References

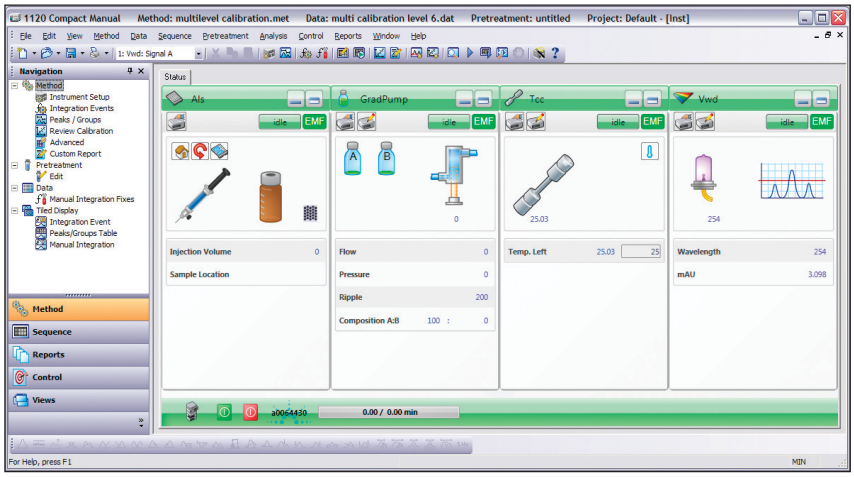
1.
X. Ping, “Analysis of preservatives in beverage using Agilent TC C18 column”, *Agilent Technologies Application Note, publication number 5989-3638CHCN,* **2005.**

2.
B. Huang, “Analysis of 8 preservatives and 3 sweeteners in sauce and beverage by HPLC”, *Chinese Journal of Health Laboratory Technology, 15(10), 1208*, **2005.**

Analysis of sweeteners in food and beverages with the Agilent 1120 Compact LC system

Application Note

Rongjie Fu,
Zhixiu Xu



Abstract

An analysis method for three of the most popular artificial sweeteners was developed in this application, and the sweeteners were analyzed in a food and a beverage. The system suitability results showed that the Agilent 1120 Compact LC is the system of choice for conventional, analytical scale liquid chromatography. This integrated HPLC system was designed for ease of use, performance, and reliability. The quantitative analysis of typical samples is demonstrated in this Application Note.

5989-8961EN

Introduction

Artificial sweeteners are widely used all over the world, and some of them have a long history. For example, saccharin was invented nearly 100 years ago. Artificial sweeteners taste similar to cane sugar, but are low-calorie. They benefit overweight people and those who have problems with sugar metabolism. Artificial sweeteners are also cheaper than natural sugar and can reduce the cost for some foods and beverages. However, scientific research has shown that some of them can cause tumors in certain animals, so to prevent potential danger to humans, it is necessary to control the amount of sweeteners in foods and beverages.

Regulations set an upper limit on the concentration of artificial sweeteners in foods and beverages. The labels of foods and beverages should list what kinds of sweeteners are used. Quality control or spot-checking can use a conventional HPLC method to determine the amount of the sweeteners in the samples.
For this application, the most widely used sweeteners were analyzed in samples of yogurt and a beverage. The analysis was performed with the Agilent 1120 Compact LC, which is the system of choice for conventional, analytical scale liquid chromatography. It is an integrated HPLC system designed for ease of use, performance, and reliability. It is ideally suited for routine analyses in the food industry because of its capability to achieve very precise retention times and peak areas, as well as low detection limits for the analyzed compounds.

Experimental

Equipment

- Agilent 1120 Compact LC system with gradient pump (degasser inside), autosampler, column compartment, and variable wavelength detector (VWD)
- EZChrom Elite Compact software

Chemicals and reagents

- Reference standards were purchased from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China.
- Water was obtained from a Millipore water purifier.
- Acetonitrile (Fisher Scientific) was HPLC purity. All other reagents were analytical purity.

Sample preparation

Yogurt: 5 mL was diluted with 5 mL methanol, and then the mixture was stirred and centrifuged. The sample was filtered with a 0.45 µm filter prior to injection.

Diet Coke: The sample was treated with an ultrasonic for 10 minutes, and then was filtered with a 0.45 µm filter prior to injection.

Chromatographic conditions

- Column: Agilent TC-C18(2), 4.6 x 250 mm, 5 µm
- Mobile phase: A = 20 mM KH_2PO_4 buffer, pH 3.0; B = acetonitrile
- Gradient: 0 min 15 %B
  5 min 35 %B
  10 min 80 %B
- Flow rate: 1 mL/min
- Wavelength: 214 nm
- Injection volume: 5 µL
- Temperature: 30 °C

Results and discussion

The separation of standards of three sweeteners (acesulfame, saccharin, and aspartame) was done in eight minutes. To make sure the other components of the real sample were eluted from the column, the final method needed 11 minutes runtime.

By overlaying the chromatograms of the standards and the real samples, one can easily find out which kind of sweeteners are used in specific samples. As shown in figure 1, the samples of yogurt and Diet Coke that were used for this test contained both acesulfame and aspartame, but no saccharin.

The linearity of the compounds was tested within the concentration range from 18.75 to 1500 ng, which covers the most likely concentrations in real samples. The results are shown in table 1. The data shows that very good regression factors (values of r^2) were achieved for each compound.

The system reproducibility was also tested with the three compounds. The high precision of the retention times gives high confidence when comparing the standards and real samples.

The quantitative results from the two samples are shown in table 3.

Conclusion

The Agilent 1120 Compact LC is ideal for the routine analysis of sweeteners in foods and beverages. Excellent resolution and good separation were achieved, and system suitability experiments showed the robustness and high precision for this kind of application. The high precision of the retention times and peak areas ensures reliable results when quantitation is needed for quality control.

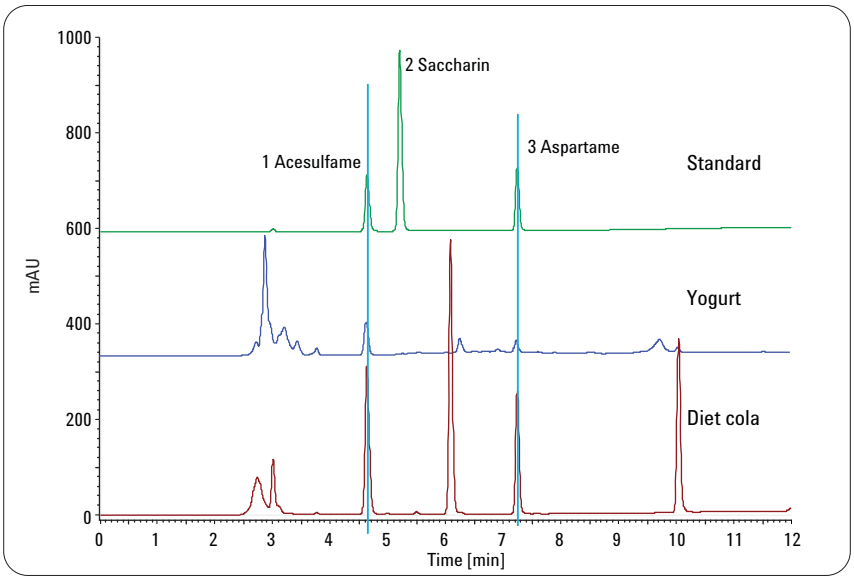


Figure 1
Overlaid chromatograms of sweetener standards and real samples.

Peak	Compound	Range (ng)	r^2
1	Acesulfame	18.75 - 1500	0.99999
2	Saccharin	18.75 - 1500	0.99997
3	Aspartame	18.75 - 1500	0.99998

Table 1
Linearity of the sweetener standards.

Peak	Compound	% RSD retention times	% RSD areas
1	Acesulfame	0.075	0.09
2	Saccharin	0.070	0.24
3	Aspartame	0.033	0.23

Table 2
Reproducibility of the 10 injections of sweetener standards.

	Acesulfame	Aspartame
Yogurt	0.09 mg/mL	0.027 mg/mL
Diet Coke	0.205 mg/mL	0.146 mg/mL

Table 3
The amount of sweeteners in the real samples.

References

1.
X. Ping, "Analysis of sweeteners in food and beverage using Agilent TC C18", *Agilent Technologies Application Note, publication number 5989-3890CHCN,* **2005.**

2.
W. Liu, "Determination of Antiseptic and Sweetener in wine by HPLC", *Liaoning Chemical Industry, 35(4), 238,* **2006.**

3.
X. Sheng, "Solid phase extraction-liquid chromatography/mass spectrometry for simultaneous determination of artificial synthetic sulfa sweeteners in food", *Chinese Journal of Analysis Laboratory, 25(7), 75,* **2006.**

Determination of preservatives in food and drugstore products with the Agilent 1120 Compact LC

Application Note

Food

Authors

Detlef Wilhelm
ANATOX GmbH & Co. KG
Fuerstenwalde
Germany

Abstract

This Application Note describes the analysis of several well known preservatives like benzoic acid, sorbic acid or the esters p-hydroxy benzoic acid (methyl-, ethyl- and propyl-) with the Agilent 1120 Compact LC.

Conventional LC methods are often used in routine analyses to characterize or monitor products for this purpose. The presented data show the determination of these ingredients in several consumer products such as chewing gum, ketchup, and barbecue sauce, which demonstrates the relevance of this method for quality control testing and monitoring of products for consumer protection.

This Application Note shows that the Agilent 1120 Compact LC works as a reliable and highly robust instrument for standard LC. It can be used for routine analyses. The use of methanol for separating the components reduces cost and allows a 15-min determination of all components with an overall resolution of >3. The results of reliability, quality, system suitability and performance testing are shown.

Introduction

Several consumer products contain preservatives, which can be harmful to some people. The most common preservatives are benzoic or sorbic acid as well as the esters p-hydroxy benzoic acid. These ingredients inhibit microbiological growth in food or drugstore products and are used either alone or as a mixture. During manufacturing, the products are analyzed for quality control and monitored for consumer protection. Standardized conventional LC methods are often used in routine analyses to characterize or monitor products.

There are several requirements for the analytical instrumentation: reliability, flexibility, and ease of use. In addition, the instrument should provide low ownership cost.

This Application Note shows that the Agilent 1120 Compact LC works as a reliable and highly robust instrument for standard LC. It can be used in routine analyses, for preservatives found in several consumer products. These analyses are done in independent control laboratories; therefore the use of methanol for separating the components should be optimized to reduce cost and pollution.

The results of reliability, quality, system suitability and performance tests are shown.

Instrumentation

An Agilent 1120 Compact LC with the following configuration was used:

Configuration of the Agilent 1120 Compact LC
Gradient pump and vacuum degasser
Auto sampler
Column oven
Variable wavelength detector
Software: EZ-Chrom Elite Compact 3.3

Preparation of samples

Reference samples

Dissolve 10 mg of each vitamin in water and dilute to 100 mL with the same solvent. Mix and dilute 1 mL of each solution to 20 mL with mobile phase.

The following common preservatives were checked:
Benzoic acid, sorbic acid, methyl-, ethyl- and propylesters of p-hydroxy benzoic acid (Methyl-PHB, Ethyl-PHB, Propyl-PHB).

Samples from food and drugstores

Dissolve 1g of sample in 10 mL of water. Solution and extraction can be improved by treating for 10 min in a ultrasonic bath. Next, filter the samples with syringe filters; first through a 2-µm filter, followed by a 0.45-µm syringe filter to prepare the clear solution for injection.

Chromatographic conditions		
Column	ZORBAX Eclipse XDB C18, 150 mm ˜ 4.6 mm, 5 µm	
Mobile phases		
Phase A:	Dissolve 6.8 g potassium dihydrogen phosphate in 900 mL water. The pH value should be adjusted to pH = 2.3 with phosphoric acid and then filled to 1000 mL with water.	
Phase B:	Methanol	
Gradient (linear):	Time (min)	
	0	80% A/20% B
	9.6	47% A/53% B
	12	33% A/67% B
	13	33% A/67% B
	13.1	80% A/20% B
Pump settings		
Stop time:	15 min	
Post time:	5 min	
Flow rate:	1.75 mL/min	
Autosampler		
Injection volume:	20 µL	
Thermostatted column compartment		
Temperature:	40 °C	
Detector	14 µL cell, Peak width: >0.05 min, 1 s response time (10 Hz), Signal: 220 nm	

System suitability and performance test:
For system suitability testing, the reference solution with the limits listed below was used. This was in accordance with Q3A(R)- Impurities in New Drug Substances:[2]

- Resolution: minimum 1.5 between each peak
- Precision of areas must be < 2 % RSD.
- Precision of retention times must be < 0.5 % RSD.

With these limits and settings for testing, the samples in Table 1 were prepared and analyzed.

Sample	Purpose	Number of injections
Blanc solution	Verify baseline stability and identify artifacts	2
Calibration samples	Verify linearity	3 of each level
Control sample	Verify sensitivity and resolution for reference solution	6
Suitability sample	Verify precision of areas and retention times for reference solution	10

Table 1
Setup for testing.

Results and discussion

The separation was achieved with methanol, since it was able to separate the critical pair of benzoic and sorbic acid.[2] The results in Figure 1 show good separation of all preservatives with the Agilent ZORBAX Eclipse XDB C18 material on the Agilent 1120 Compact LC and EZChrom Elite Compact Software.

For the detection of all components, the Agilent 1120 Compact LC variable wavelength detector was set for 260 nm. This setting was chosen because all target analytes except benzoic acid has a maximum adsorption near 260 nm.

Detailed data are listed in Table 2. The first limit for resolution (>1.5) is fulfilled for all peaks. The data for resolution show good selectivity with the ZORBAX

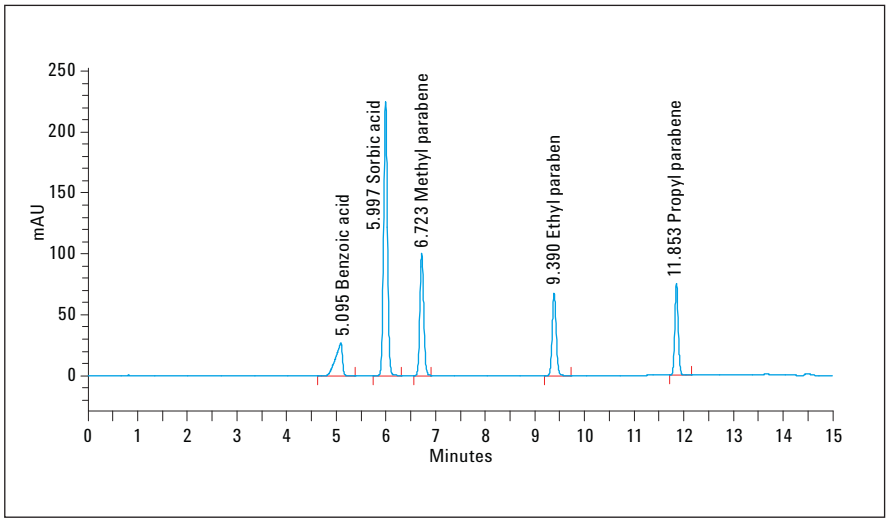


Figure 1
Standard chromatogram of preservatives with the Agilent 1120 Compact LC.

Compound	Retention time (min)	Resolution
Benzoic acid	5.06	–
Ascorbic acid	5.96	4.31
Methyl-PHB	6.69	5.25
Ethyl-PHB	9.39	18.55
Propyl-PHB	11.85	17.83

Table 2
Results for control sample: Retention times and resolution.

Eclipse XDB C18 material.

The areas and retention time precision results of all compounds of the suitability sample are shown in Table 3. The data demonstrate the high reliability and precision of the Agilent 1120 Compact LC. The data show that the system can be used for QC methods, since the criteria for retention times and areas are fulfilled for all compounds.

The data in Tables 3 and 4 prove high precision and reliability of the autosampler. The correlation coefficient for each calibration curve is very close to 1.0 showing high versatility and quality for QC testing and monitoring.

It can be seen that with the selected column, as well as with the Agilent 1120 Compact LC, characterization and monitoring of products from foods and drugs is possible. The following examples demonstrate the performance of the system:

• The chromatogram of Figure 2 illus-

Compound	Retention time (min)	RSD RT n = 10	RSD Area n = 10	Asymmetry n = 10
Benzoic acid	5.06	0.408	0.188	0.64
Ascorbic acid	5.96	0.153	0.235	1.00
Methyl-PHB	6.69	0.277	0.260	1.09
Ethyl-PHB	9.39	0.039	0.112	1.09
Propyl-PHB	11.85	0.009	0.074	1.10

Table 3
Suitability sample: Precision of retention times and areas for the Agilent 1120 Compact LC.

Compound	m	b	r
Benzoic acid	2151314.8	25187.3	0.999
Ascorbic acid	10188005.0	119381.1	1.0
Methyl-PHB	4459268.1	83135.1	1.0
Ethyl-PHB	3097910.6	57372.8	1.0
Propyl-PHB	3024691.2	24203.6	1.0

Table 4
Calibration data of the Agilent 1120 Comapct LC (Setting "Ignore Origin", Y = mx + b, 1.0 µg/mL–20.0 µg/mL.)

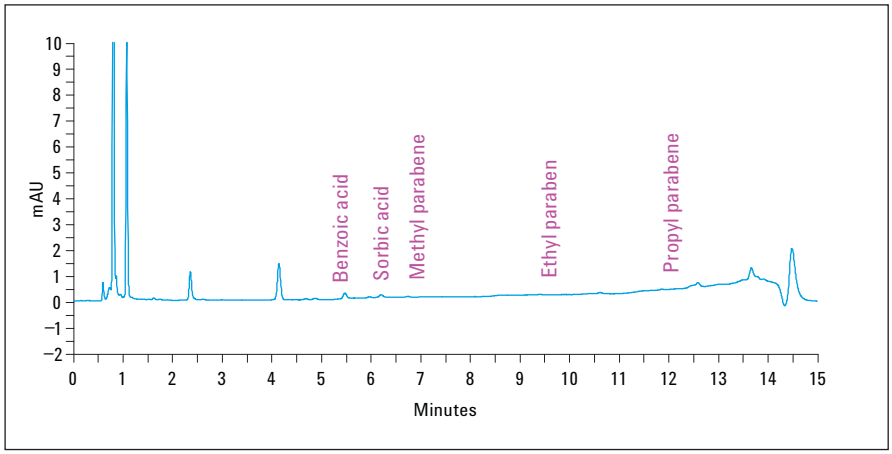


Figure 2
Analysis of preservative "free" chewing gum.

trates that the chewing gum is free of all of the preservatives tested.

- The chromatogram of Figure 3 shows that the mouthwash tested is free of preservatives tested, but it is possible that different preservatives were used to inhibit microbial growth.
- The chromatograms in Figures 4 and 5 show the analysis of a beauty cream and a toothpaste. The declared preservative (Methyl-PHB) is part of the product. This chromatogram verifies

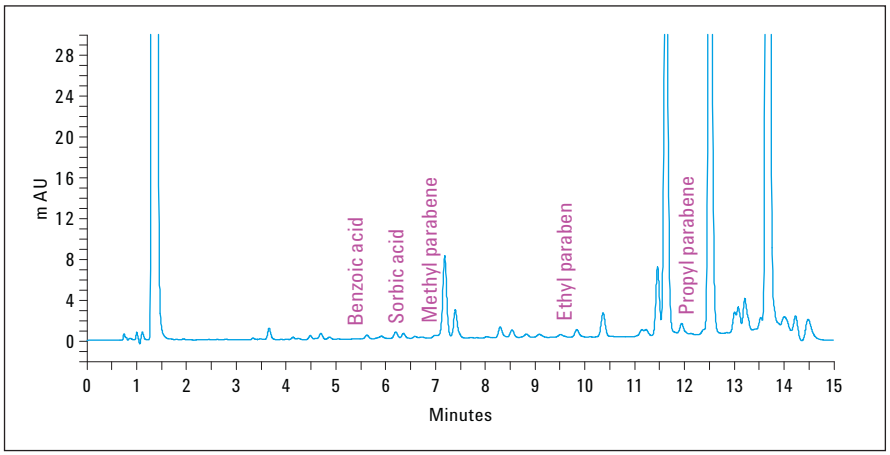


Figure 3
Analysis of mouthwash.

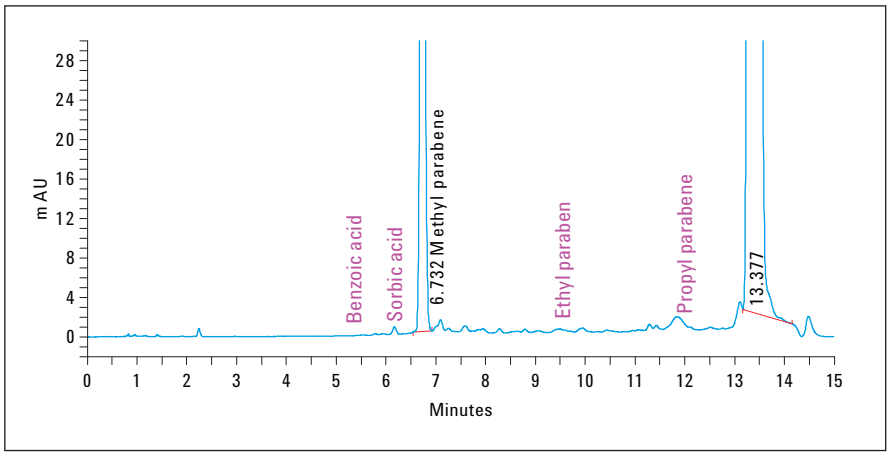


Figure 4
Chromatogram of a beauty cream.

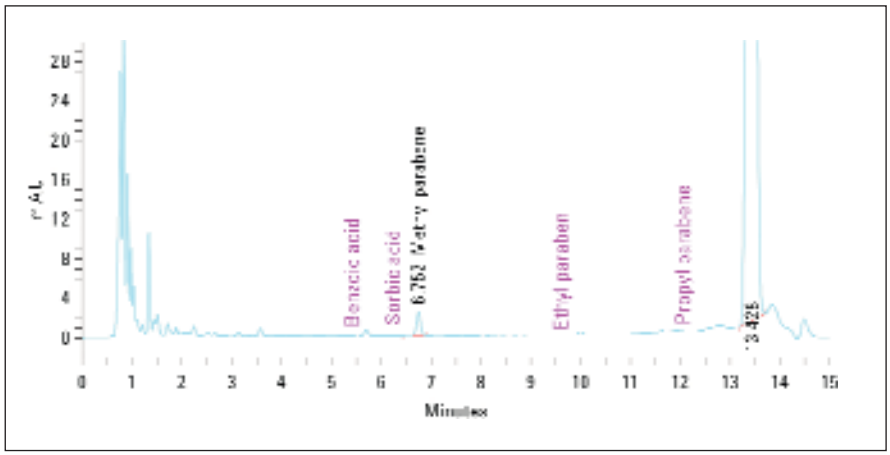


Figure 5
Chromatogram of a toothpaste.

that the method can be used for quality control as well as for monitoring.

- The chromatograms in Figures 6 and 7 show the method can monitor supermarket products. The preservative-free brand of ketchup is proven to be free of the tested preservatives. The preservative-free barbecue sauce contains some considerable amounts of benzoic acid and methyl-PHB.

All examples show minimal influence of the matrix on the separation. It can be seen that it is easy to detect any of the tested preservatives.

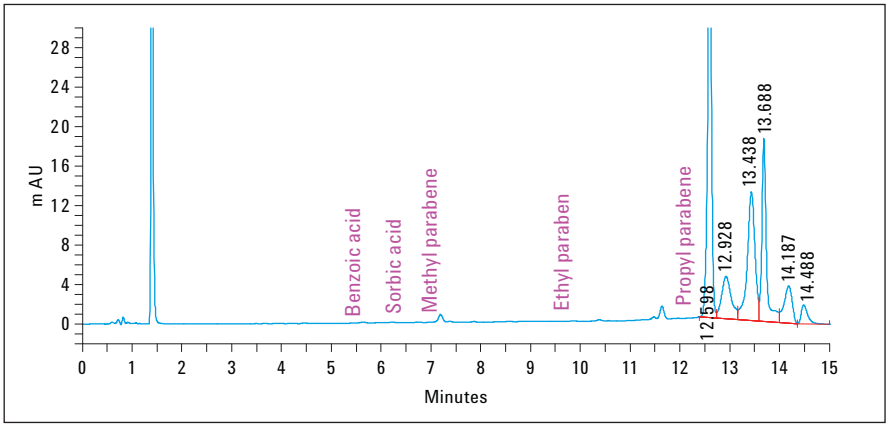


Figure 6
Analysis of a "preservative free" ketchup brand.

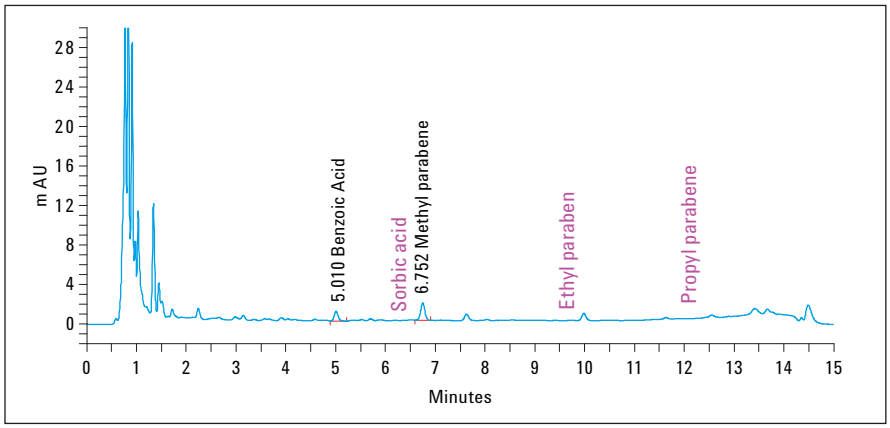


Figure 7
Analysis of a "preservative free" barbecue sauce.

Conclusion

The Agilent 1120 Compact LC is designed for users in independent labs who require LC methodology with reliability, ease-of-use, and low ownership cost, to characterize or monitor products.

This Application Note shows a reliable approach for the determination of preservatives in food or drugstore products. The data prove high precision of retention time, and provide clear chromatographic parameters such as resolution and asymmetry.

As shown in Table 2, the resolution of all main peaks was found to be greater than 3.0. The calibration data of each compound shows that the instrument can be operated as required in a quality control environment. All criteria for precision, such as areas and retention times are fulfilled (see Table 3). The results indicate that the use of the Agilent 1120 Compact LC in QA/QC laboratories to characterize products containing preservatives is appropriate. In addition, it is possible to identify any of the tested preservatives independent of the matrix.

All results explicitly show the applicability of the Agilent 1120 Compact LC for quality control testing with reduced costs per system and improved simplicity of use. In addition to the instrument capabilities, the new version of the EZChrom Elite compact software allows full control of the Agilent 1120 Compact LC with a wide range of features for data analysis and results reporting.

The results for resolution and asymmetry show good selectivity and performance with the ZORBAX Eclipse XDB.

The Agilent 1120 Compact LC is qualified and optimized for everyday productivity and routine analysis.

References

1. "Analysis of Preservatives in White Wine an Salad Dressing using HPLC," 1997, Agilent Technologies publication 5966-0629E

2. Q3A(R) Impurities in New Drug Substances, Rev. 2, http://www.fda.gov/cder/guidance/7838fnl.pdf,

Determination of water soluble vitamins with the Agilent 1120 Compact LC after method development with the Agilent 1200 Series Rapid Resolution LC system and back transfer

Application Note

Food

Author

Detlef Wilhelm
ANATOX GmbH & Co. KG
Fuerstenwalde
Germany

Abstract

Quality control to characterize products is often done with standardized liquid chromatography (LC) methods. The needed analytical instrumentation requires optimal cost-of-ownership instruments with high reliability, high flexibility, and ease of use.

The analysis of several water soluble vitamins with the Agilent 1120 Compact LC is described. The preceding method development was done with Agilent 1200 Series Rapid Resolution (RR). This Application Note starts with the final results of that method development. The transfer of those results by the Method Translator Software to conventional LC parameters and columns shows that this method can be used with standard LC equipment.

This Application Note shows that the Agilent 1120 Compact LC works as a reliable and highly robust instrument for routine LC analyses and everyday quality control testing. Full separation was achieved by using an ion pairing eluent and Agilent ZORBAX Eclipse Plus material. The analysis of a typical nutritional mixture of vitamins shows no disturbances by other peaks. The results for reliability and quality testing, system suitability, and performance are shown.

Introduction

Quality control is a main consideration in the field of standard LC product analyses. The analytical instrumentation has several requirements, such as optimal cost-of-ownership, high reliability, high flexibility, and ease of use.

This Application Note targets routine quality control testing, and shows that the Agilent 1120 Compact LC is a reliable and highly robust instrument for standard methodology. The Agilent 1120 Compact LC can also be used for determinations after method development with an Agilent 1200 Series RRLC system. Back transfer from the RR separation to conventional columns can be achieved by the Method Translator Software, and the results of reliabiltiy, quality, system suitability, and performance testing are shown.

Instrumentation

An Agilent 1200 Series Rapid Resolution LC system and an Agilent 1120 Compact LC were used for the method development. Table 1 lists the configurations used for each instrument.

Configuration of the Agilent 1200 LC Series	Configuration of the Agilent 1120 Compact LC
Binary pump and vacuum degasser	Gradient pump and vacuum degasser
Well-plate autosampler	Autosampler
Column compartment	Column oven
Diode array detector	Variable wavelength Detector
Software: Chemstation B.04.01	Software: EZ-Chrom Elite Compact 3.3

Table 1
Instrumentation configurations used for method development

Preparation of samples

Reference samples

Dissolve 10 mg of each vitamin in water and dilute to 100 mL with the same sol vent. Dilute 1 mL of the solution to 20 mL with the mobile phase. Figure 1 illustrates the substances to check.

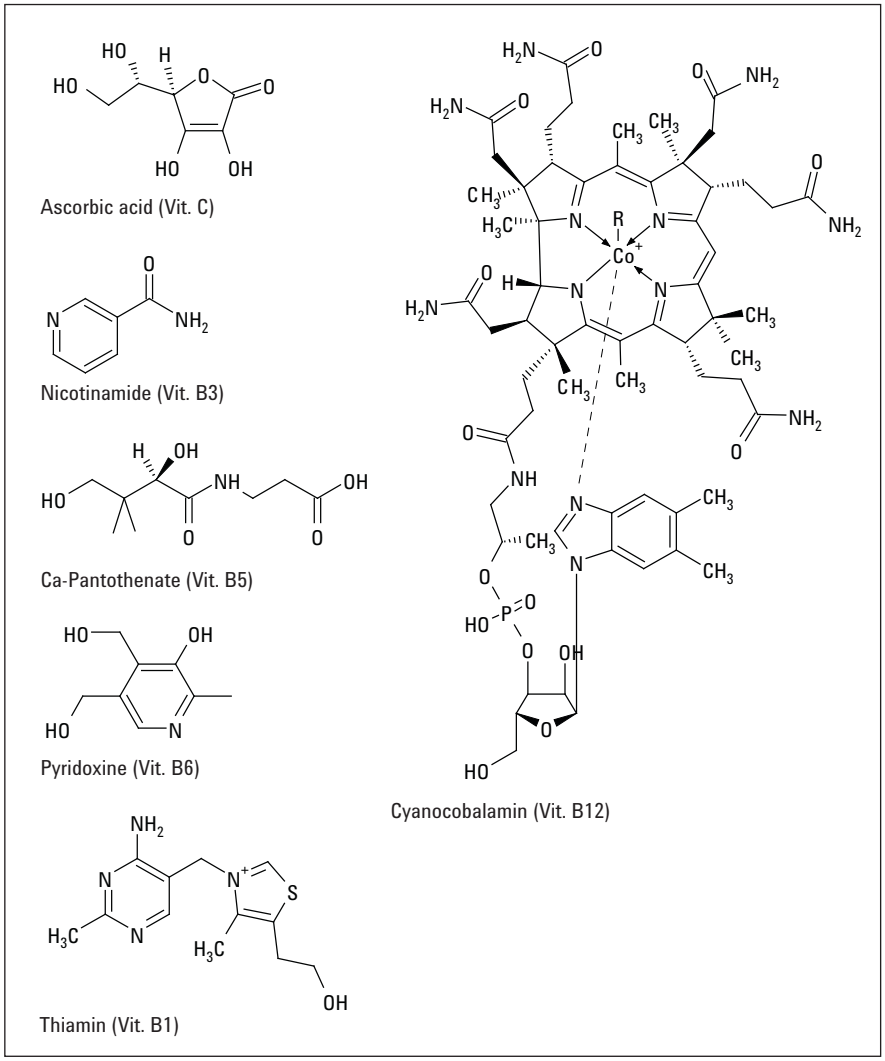


Figure 1
Substances to check.

Chromatographic conditions		
Column		
For method development:	ZORBAX Eclipse Plus C18, 50 mm ˘ 2.1 mm, 1.8 µm	
For routine testing:	ZORBAX Eclipse Plus C18, 150 mm ˘ 4.6 mm, 5 µm	
Mobile phases		
Phase A:	Dissolve 1.03 g hexane sulfonic acid and 6.8 g potassium dihydrogene phosphate in 1000 mL water. The pH value should be adjusted to pH = 2.3 with phosphoric acid.	
Phase B:	Acetonitrile	
Gradient (linear):	Time (min)	
	0	100% A/0% B
	6	80% A/20% B
	9	50% A/50% B
	10	100% A/0% B
Pump settings		
Stop time:	10 min	
Post time:	5 min	
Flow rate:	1.0 mL/min	
Autosampler		
Injection volume:	30 µL	
Thermostatted column compartment		
Temperature:	40 °C	
Detector	14-µL cell for the Agilent 1120 LC system, Peak width: >0.05 min, 1 s response time (10Hz), Signal: 220 nm 13-µL cell for the Agilent 1200 LC system, Peak width: >0.05 min, 1 s response time (10Hz), Signal: 220 nm	

System suitability and performance test:

For system suitability testing the reference solution with the following limits was used:

- Resolution: minimum 1.5 between peaks.
- Precision of areas must be < 2 % RSD.
- Precision of retention times must be < 0.5 % RSD.

With these limits and settings for testing, the samples in Table 2 were prepared and analyzed.

Sample	Purpose	Number of injections
Blanc solution	Verify baseline stability and identify artifacts	2
Calibration samples	Verify linearity	3 of each level
Control sample	Verify sensitivity and resolution for reference solution	6
Suitability sample	Verify precision of areas and retention times for reference solution	10

Table 2
Setup for testing

Results and discussion

Hexanesulfonic acid was chosen as the ion-pairing agent to achieve the best separations for all peaks. The results in Figure 2 show good separation of all vitamins with the ZORBAX Eclipse Plus C18 material on an Agilent 1200 Series RRLC system.

The parameters found during method development with the Agilent 1200 Series RRLC system were transferred to parameters suitable for using 5-µm columns with conventional HPLC systems with the Agilent Method Translator Software (see Figure 3).

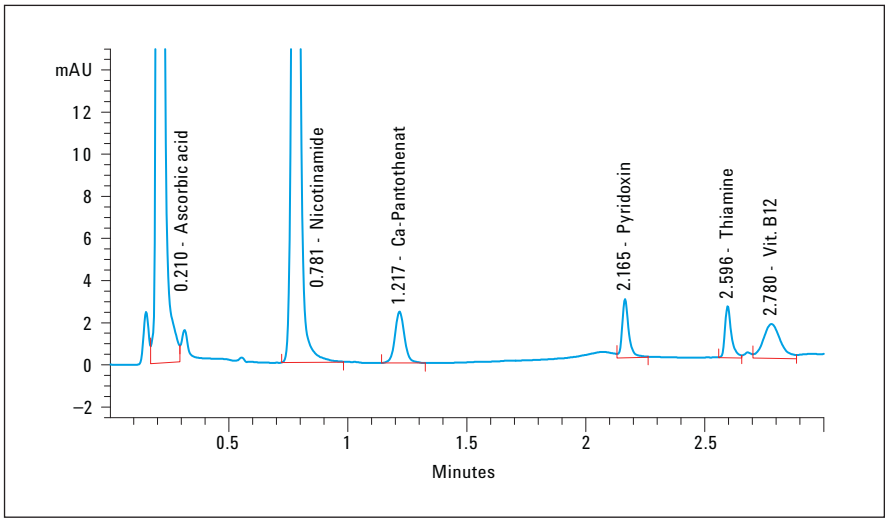


Figure 2
Example chromatogram of water soluble vitamins with the Agilent 1200 Series RRLC system.

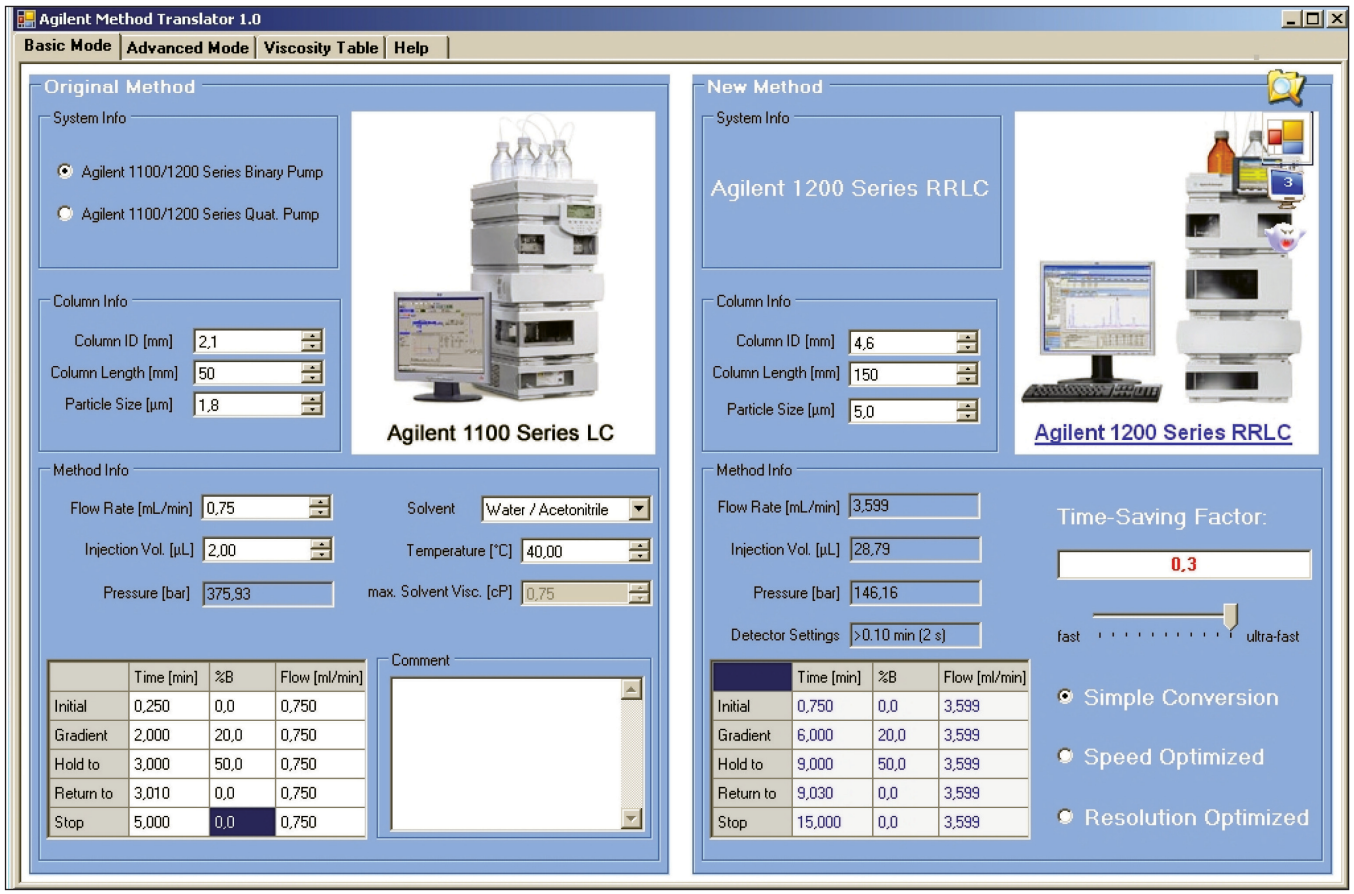


Figure 3
Conversion of LC parameters found by Rapid Resolution to parameters suitable for conventional HPLC with the Method Translator software.

Because Ca-pantothenate has no significant absorbance at wavelengths >230 nm, a 220 nm setting on the Agilent 1120 Compact LC wavelength detector was selected to detect all components. Figure 4 shows a chromatogram, achieved after transfer of the parameters from the 50 mm ˘ 2.1 mm Rapid Resolution column to a 150 mm ˘ 4.6 mm (5-µm material) column with the Agilent 1120 Compact LC and EZ-Chrom Elite Compact Software.

As Figure 4 shows, the elution order is the same, which proves that the selectivity does not change with the particle size. Only small differences can be seen in the resolution between nicotinamide and Ca-pantothenate, but full baseline separation still exists. Detailed data are listed in Table 3. As a main result it can be emphasized that it is possible to transfer LC parameters from Rapid Resolution to conventional LC systems and columns with 5-µm particles.

The only difference in parameters between the Agilent 1200 Series binary system and the Agilent 1120 Compact LC is that the 1120 LC separation resulted in slightly later eluting peaks, as seen in Figures 2 and 3. The reason can be found in the difference of gradient mixing. With the Agilent 1120 Compact LC, the gradient is mixed at the low pressure site whereas with the Agilent 1200 binary system the mixing is done at the high pressure site. The different mixing volumes (delay volumes) are responsible for the different raise of the gradients in the columns.

However, as seen in Figure 3 the resolution of all peaks is unaffected by the different gradient mixing. In addition, all other impurities in the fine chemicals are separated.

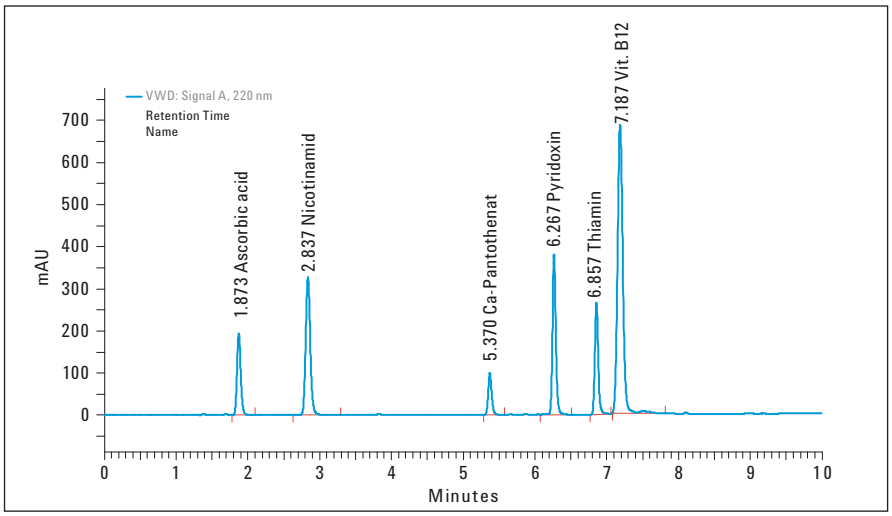


Figure 4
Example chromatogram of water soluble vitamins with the Agilent 1120 Compact LC.

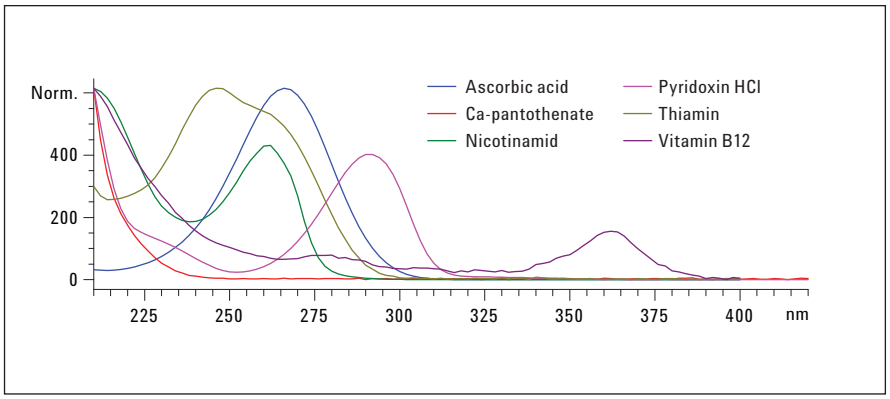


Figure 5
UV spectra of all vitamins aquired by the Agilent 1200 Series Diode Array Detector.

Compound	Retention time 1200er (min)	Retention time 1120 (min)	Resolution 1200er	Resolution 1120
Ascorbic acid	0.210	1.873	–	–
Nicotinamide	0.781	2.837	12.78	9.24
Ca-Pantothenate	1.217	5.370	7.27	23.18
Pyridoxine	2.165	6.267	16.68	10.10
Thiamine	2.596	6.857	9.76	6.83
Vitamin B12	2.780	7.187	2.24	3.38

Table 3
Results for control sample: Retention times and resolution.

The results shown in Table 3 illustrate good separation achieved with the ZORBAX Eclipse Plus C18 material. The high resolution (every peak >2) for both systems shows that this column material is highly suitable for this determination, because of its good performance (see data for asymmetry in Table 4).

Table 4 shows the areas and retention time precision results of all compounds in the suitability sample. The reliability and precision of the Agilent 1120 Compact LC is demonstrated. The criteria (see "System suitabiltiy and performance tests") of precision of retention times and areas are fulfilled for all compounds. This shows that the system can be used for QC methods.

The high precision and reliability of the autosampler is best shown by the data in Tables 4 and 5. The correlation coefficient for each calibration curve is very close to 1.0 showing high versatility and quality for QC testing.

The chromatogram in Figure 6, shows the determination of a nutritional mixture of vitamins with great differences in concentration of each vitamin, which illustrates the capabilities of the method.

Compound	Retention time (min)	RSD RT n = 10	RSD Area n = 10	Asymmetry 1120
Ascorbic acid	1.873	0.225	0.234	1.186
Nicotinamide	2.837	0.398	0.236	1.185
Ca-Pantothenate	5.370	0.277	0.508	1.238
Pyridoxine	6.267	0.323	0.325	1.178
Thiamine	6.857	0.407	0.374	1.193
Vitamin B12	7.187	0.145	0.652	1.116

Table 4
Suitability sample: Precision of Retention times and areas for the Agilent 1120 Compact LC.

Compound	m	b	r
Ascorbic acid	1601035.9	48890.7	0.999
Nicotinamide	17812069.2	17303.5	1.0
Ca-Pantothenate	2231477.8	3546.1	1.0
Pyridoxine	35097452.7	75231.6	0.999
Thiamine	26988637.8	40978.7	0.999
Vitamin B12	104653435.3	19666.3	0.999

Table 5
Calibration data of the Agilent 1120 Comapct LC (Setting "Ignore Origin", Y = mx + b, 1.6–16.1 µg/mL for ascorbic acid and 0.11–1.12 mg/L for the other vitamins).

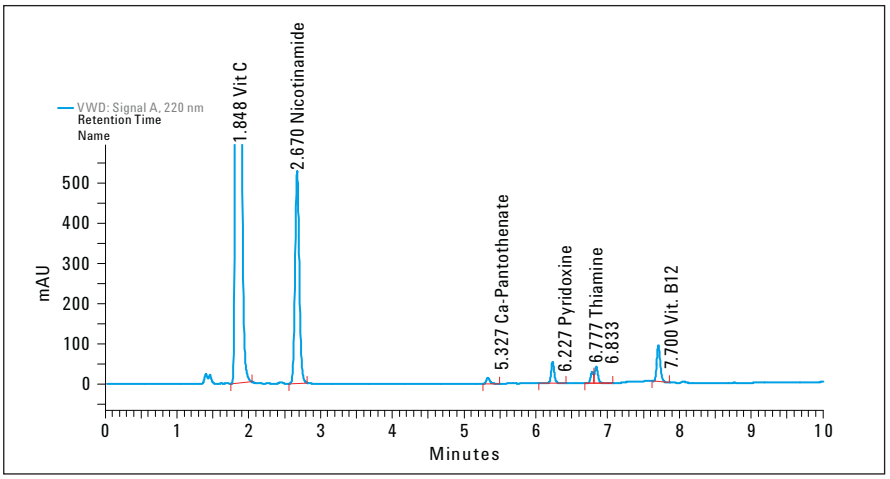


Figure 6
Chromatogram of a typical nutritional mixture of vitamins.

Conclusion

The Agilent 1120 Compact LC is a good choice for medium to small sized company users who need high reliability, ease-of-use, and lowest cost-of-ownership for standard QA/QC LC methodology.

This Application Note shows the setup for the determination of water soluble vitamins after method development with rapid resolution. This reliable approach is proven with precision of areas and retention time data, as well as chromatographic parameters such as resolution and asymmetry. The results from a system optimized for everyday productivity and calibration meet the requirements for routine analysis.

As shown in Table 3 the resolution of all peaks was found to be greater than 3.0 with values near 1.0 for the asymmetry of all main peaks. It is concluded from the calibration data that the instrument can be operated in a quality control environment.

The results in Table 4 show that all criteria for the determination of precision (areas, retention times) are fulfilled. From these results, it can be concluded that the Agilent 1120 Compact LC can be used in QA/QC laboratories to determine water soluble vitamins in samples for nutritional purposes.

All results show the applicability of the Agilent 1120 Compact LC for quality control testing by reduced costs per system and improved simplicity of use. In addition to the instrument capabilities, the new version of the EZChrom Elite compact software allows full control of the Agilent 1120 Compact LC with a wide range of features for data analysis and results reporting.

The results for resolution and asymmetry show good selectivity and performance of the ZORBAX Eclipse Plus material, independent of the particle size. The data also show good flow design of the LC systems ensuring that no band broadening and peak distortion will occur during method transfer. In summary, this Application Note shows that fast method development can be achieved with an Agilent 1200 Series Rapid Resolution LC system and the results can be back transferred to a conventional HPLC such as the Agilent 1120 Compact LC. This approach will meet the highest requirements for everyday productivity.

Quantitative liquid chromatography analysis of melamine in dairy products using Agilent's 1120 Compact LC and 1200 Series Rapid Resolution LC

Application Note

Food Safety

Authors

Hua Wu, Rong An, Yao Xiao, Zhixu Zhang, and Ping Li
Agilent Technologies Co., Ltd.
3 Wang Jing Bei Lu,
Beijing 100102
China

Editor

Wei Luan
Agilent Technologies Co., Ltd.
412 Ying Lun Road
Shanghai 200131
China

Abstract

In this application, three different LC methods are developed for the determination of melamine in dairy products. The first is a modification of the U.S. FDA method [1]. An Agilent LC system (1120 or 1200) is used with a ZORBAX SB-C8 LC column to run in reversed-phase ion-pair mode for routine quantitation of melamine. The second method is targeted for high throughput using an Agilent Rapid Resolution LC (RRLC) system (1200SL) to speed melamine analysis by more than three times with a Rapid Resolution High Throughput (RRHT) column. The third is an alternative ion-exchange LC method where a ZORBAX 300SCX column is employed to successfully retain melamine using a simple mobile phase of buffered water/acetonitrile without the presence of ion-pair reagent. Due to the complexity of dairy product matrices, a cleanup step using solid phase extraction (SPE) is required for the above methods. The Agilent SampliQ SCX, a mixed-mode polymer SPE cartridge with combined reversed-phase and strong cation exchange properties, is used to successfully remove matrix interferences.

Introduction

In March 2007, imported pet food ingredients contaminated with melamine caused renal failure in dogs and cats across the United States. Once again, this compound is in the news as an illicit adulterant in milk and milk products. The same contaminant is now being detected in other food products that contain milk imported from China and as global concern rises, widespread testing for melamine is proceeding.

The published analytical approaches include LC, LC/MS, and GC/MS. The LC method is being used for quantitative analyses of melamine. Liquid chromatographic separation of this small polar compound can be achieved by reversed-phase ion-pair liquid chromatography. The U.S. FDA developed this methodology for melamine in pet food in 2007. With a slight modification of the proportion of mobile phase, the method can be successfully applied to separate melamine from a variety of dairy product matrices.

The disadvantage of conventional HPLC is time and solvent consumption. The Agilent 1200 Series Rapid Resolution LC system is designed for highest throughput without loss of resolution or with better resolution in combination with the Agilent ZORBAX RRHT columns. In this application note, the conventional HPLC method is transferred from a 4.6 mm ˘ 250 mm, 5 µm ZORBAX SB-C8 column to a 4.6 mm ˘ 50 mm, 1.8 µm RRHT ZORBAX SB-C8 column with equivalent results, and the LC run time is shortened from almost 20 minutes to 6 minutes.

An alternative approach for liquid chromatographic separation of this small polar compound is ion exchange chromatography. Agilent ZORBAX 300SCX is an ionic bonded-phase column packing used for cation exchange high-performance liquid chromatography. This packing consists of an aromatic sulfonic acid moiety covalently bonded to ZORBAX porous silica. This column is successfully applied to retain melamine using a simple mobile phase of buffered water/acetonitrile without the presence of ion-pair reagent.

For complex dairy product matrices, it is necessary to remove interferences such as protein, sugar, and fat before LC injection. Solid-phase extraction (SPE) is a simple way to clean up the complex matrix extract. SampliQ is a new family of SPE cartridges from Agilent with a wide range of sorbent chemistries. Among this family, the mixed-mode SampliQ Strong Cation Exchange (SCX) cartridge is a sulfonic acid-modified divinyl benzene polymer with both ion exchange and reversed-phase retention properties. This makes the SampliQ SCX very effective for cleanup after solvent extraction.

Experimental

Standard Preparation

A stock solution of melamine at 1,000 µg/mL is prepared in methanol by sonication. Dilutions in mobile phase are made up at 0.05, 0.1, 0.5, 1.0, 5.0, 10.0, 50.0, and 100.0 µg/mL concentrations.

Sample Preparation

The sample preparation process is a modification of the China national standard [2].

Sample Extraction Procedure

For liquid milk, milk powder, yogurt, ice cream, and creamy candy samples:

- Weigh 2 ± 0.01 g of sample and add to a 50-mL centrifuge tube, add 15 mL of 5% trichloroacetic acid in water and 5 mL of acetonitrile, then cap.
- Sonicate for 10 min and then place samples on vertical shaker for 10 min. Centrifuge for 10 min at 4000 rpm.
- Wet filter paper with 5% trichloroacetic acid in water, then filter the supernatant into a 25.0-mL volumetric flask, and bring to volume with 5% trichloroacetic acid in water.
- Transfer a 5.0-mL aliquot of the extract into a glass tube, and then add 5.0 mL purified water. Vortex to mix thoroughly.

For cheese, cream, and chocolate samples:

- Weigh 2 ± 0.01 g of sample, grind with 8~12 g of sea sand in a mortar, and then transfer into a 50-mL centrifuge tube.
- Wash the used mortar with 5 mL of 5% trichloroacetic acid in water three times, transfer washings into a 50-mL centrifuge tube, and then add 5 mL of acetonitrile.
- Proceed with the sonication and other steps as described in the previous preocedure..
- If the sample is very fatty, defat the extract using liquid-liquid extraction with hexane saturated with 5% trichloroacetic acid in water before cleanup by SPE.

Sample Cleanup Procedure

A SampliQ SCX SPE cartridge (p/n 5982-3236, 3 mL, 60 mg, or p/n 5982-3267, 6 mL, 150 mg) can be used to clean up sample extracts; the latter is used in this application note. All SPE elution steps, including conditioning, sample load, washing, and the final elution, are performed with a flow rate of less than 1 mL/min except for drying the cartridge by applying vacuum.

- Condition the SPE cartridge with 5 mL of methanol followed by 6 mL of water.
- Load the above sample extract to the conditioned cartridge. Wash the cartridge with 5 mL of water followed by 5 mL of methanol.
- Dry the cartridge by applying vacuum, and then elute with 5 mL of 5% ammonium hydroxide in methanol.
- Evaporate the eluate to dryness under a stream of nitrogen at approximately 50 °C.
- Reconstitute the dried extract in 1.0 mL of mobile phase, vortex for 1 min, and filter through a 0.2-µm regenerated cellulose membrane filter (p/n 5064-8222) into a glass LC vial.

Instrumentation and Conditions

Conventional HPLC method using 1120 Compact LC or 1200 LC:

- Agilent 1120 Compact LC system with gradient pump (degasser inside), autosampler, column compartment, and variable wavelength detector (VWD) or equivalent 1200 Series components
- EZChrom Elite Compact software or ChemStation software (Ver. B.04.01 or later)

Column	ZORBAX SB-C8 (also known as ZORBAX Rx-C8), 4.6 mm ˜ 250 mm, 5 µm (p/n 880975-906)
Buffer	10 mM citric acid, 10 mM sodium octane sulfonate, adjusted to pH 3.0
Mobile phase	92:8 buffer:acetonitrile
Flow rate	1.5 mL/min
Injection volume	20 µL
Column temperature	30 °C
Detection wavelength	240 nm
Run time	20 min

High-Throughput Method Using 1200SL RRLC:

- Agilent 1200SL Series binary pump, degasser, wellplate sampler, thermostatted column compartment and diode array detector (DAD)
- ChemStation software (Ver. B.04.01 or later)

Column	ZORBAX SB-C8 RRHT, 4.6 mm ˜ 50 mm, 1.8 µm (p/n 827975-906)
Buffer	10 mM citric acid, 10 mM sodium octane sulfonate, adjusted to pH 3.0
Mobile phase	92:8 buffer:acetonitrile
Flow rate	1.5 mL/min
Injection volume	8 µL
Column temperature	30 °C
Detection wavelength	240 nm
Run time	6 min

Ion Exchange Chromatography Method with 1120 Compact LC or 1200 LC:

- Agilent 1200 Series binary pump, degasser, wellplate sampler, thermostatted column compartment and variable wavelength detector (VWD) or equivalent 1120 Series components
- EZChrom Elite Compact software or ChemStation software (Ver. B.04.01 or later)

Column (p/n 883952-704)	ZORBAX 300SCX, 4.6 mm ˜ 150 mm, 5 µm
Buffer	50 mM ammonium formate solution, adjust to pH 3.0 with formic acid
Mobile phase	15:85 buffer:acetonitrile
Flow rate	1.0 mL/min
Injection volume	10 µL
Column temperature	30 °C
Detection wavelength	240 nm
Run time	5.5 min

Results and discussion

Separation of Melamine in Dairy Products by Reversed-Phase Ion-Pair LC

Melamine is not retained by reversed-phase LC and thus elutes with the solvent and unretained matrix interferences. However, using an ion-pairing reagent with reversed-phase chromatography, melamine can be well retained and separated from interferences. Figure 1 (a) is the chromatogram of melamine standard by reversed-phase ion-pair LC. Figure 1 (b) is the chromatogram of a positive yogurt sample after cleanup with the Agilent SampliQ SCX SPE cartridge.

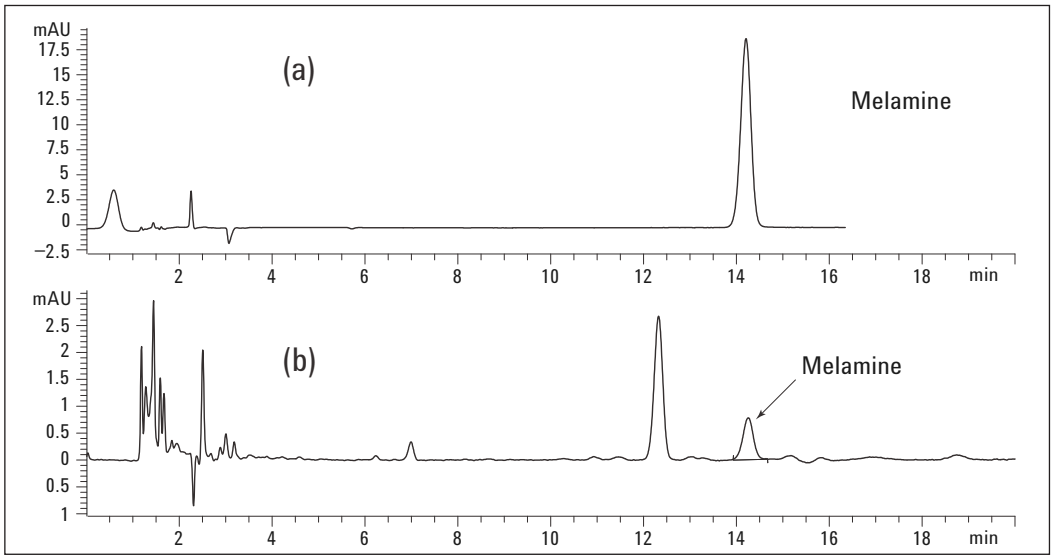


Figure 1
Separation of (a) 20 µg/mL melamine standard, and (b) positive yogurt sample after cleanup by SampliQ SCX SPE cartridge. Retention time of melamine is 14.2 minutes.

High-Throughput Analysis by Agilent 1200SL RRLC with RRHT Column

With the Agilent 1200 Series RRLC system, high throughput is possible. In combination with the Agilent ZORBAX RRHT columns, excellent chromatographic resolution can be achieved at much shorter run times than with a conventional LC system. A RRLC method is developed to dramatically increase the sample throughput for the determination of melamine in dairy products. Figure 2 (a) is the chromatogram of a melamine standard by the RRLC method with the retention time of melamine at 2.8 minutes.

Figure 2 (b) is the chromatogram of the same yogurt sample in Figure 1 (b). In order to make sure that the column is clear for the next injection, the total run time is extended to 6 minutes. The high throughput RRLC method is applied in the variety of dairy products matrices, including yogurt, liquid milk, and milk powder to demonstrate that the same resolution is achieved as with the conventional HPLC method. The calibration curve for the RRLC method is shown in Figure 3. The calibration includes 0.8, 2.0, 20.0, 40.0, and 80.0 µg/mL. The instrumental LOD (limit of detection) of the RRLC method is 0.03 µg/mL.

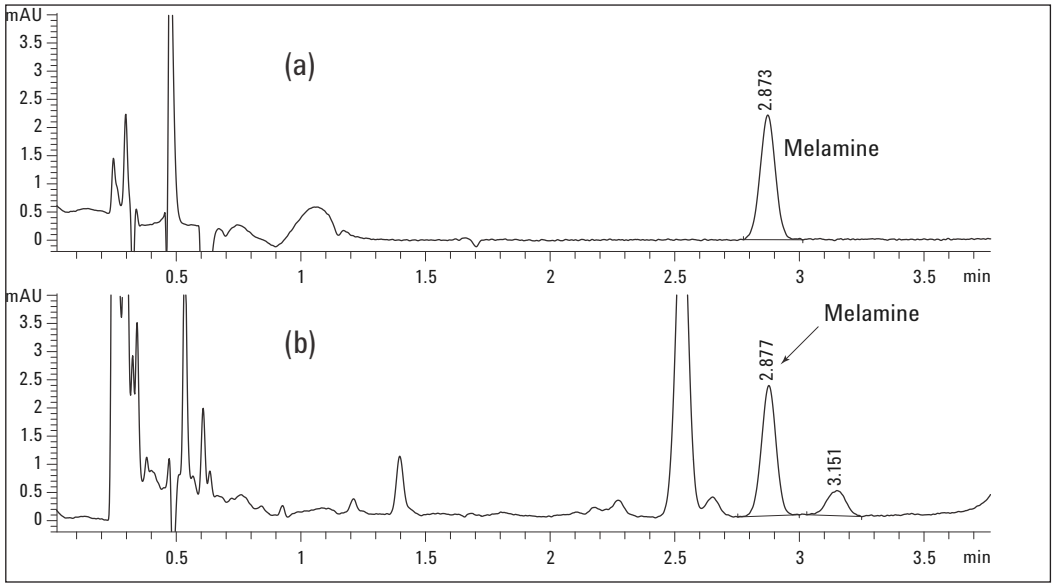


Figure 2
Separation of (a) 0.8 µg/mL melamine standard, and (b) positive yogurt sample after cleanup by SampliQ SCX SPE cartridge. Retention time of melamine is 2.8 minutes.

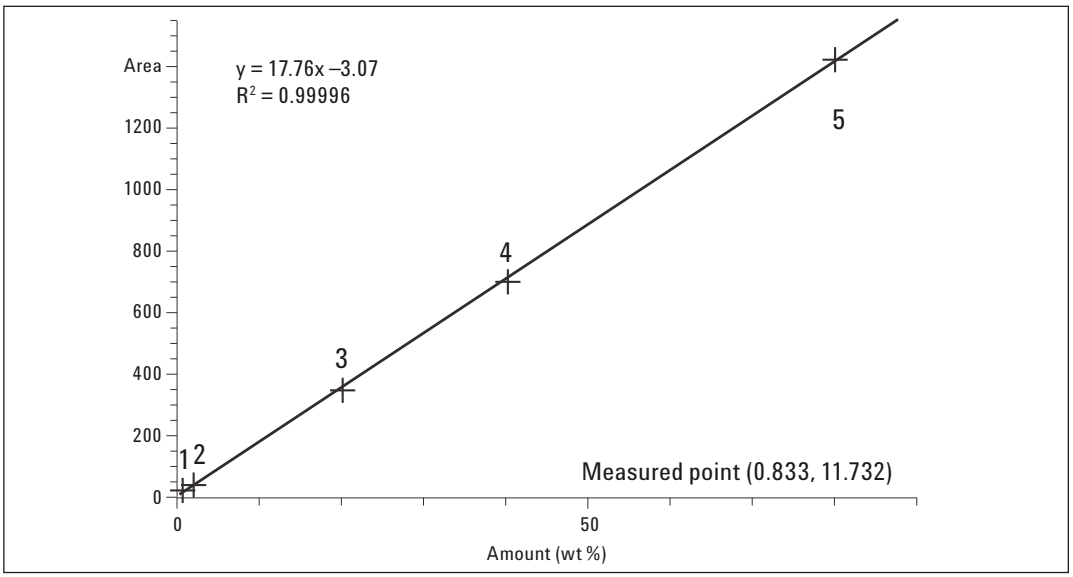


Figure 3
Calibration curve of RRLC method.

Ion Exchange Chromatographic Method

An alternative to ion-pair reversed-phase chromatography for melamine is ion exchange chromatography (IEC). The Agilent ZORBAX 300SCX is used for cation exchange high-performance liquid chromatography (HPLC). This column is employed to separate melamine in dairy product matrices with sufficient retention to separate matrix interferences. Figure 4 shows the separation of melamine from interferences without the SPE cleanup step. Generally, the total run time of the ion exchange chromatography is only 5.5 minutes with an LOD of 0.05 µg/mL, as shown in Figure 5. The calibration curve for the IEC method is shown in Figure 6. The calibration points include 0.5, 1.0, 5.0, 10, 50, and 100 µg/mL. Although the separation is shown to be interference free for raw milk and liquid milk without any additive, it is still recommended that the cleanup step be included to ensure robust methodology for running many samples and samples of different matrices.

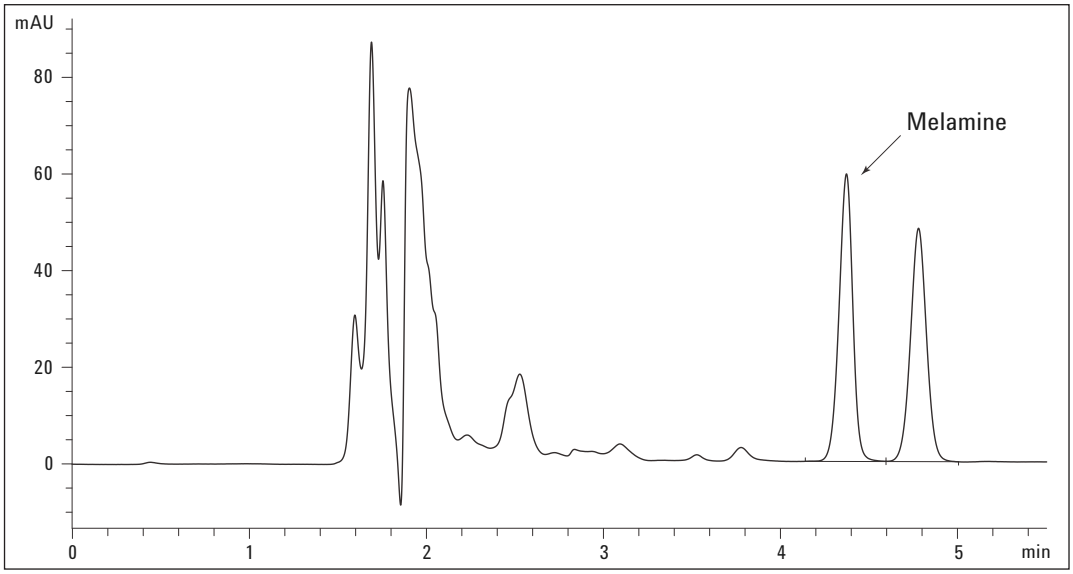


Figure 4
Separation of melamine in milk powder sample by IEC without cleanup by SPE.

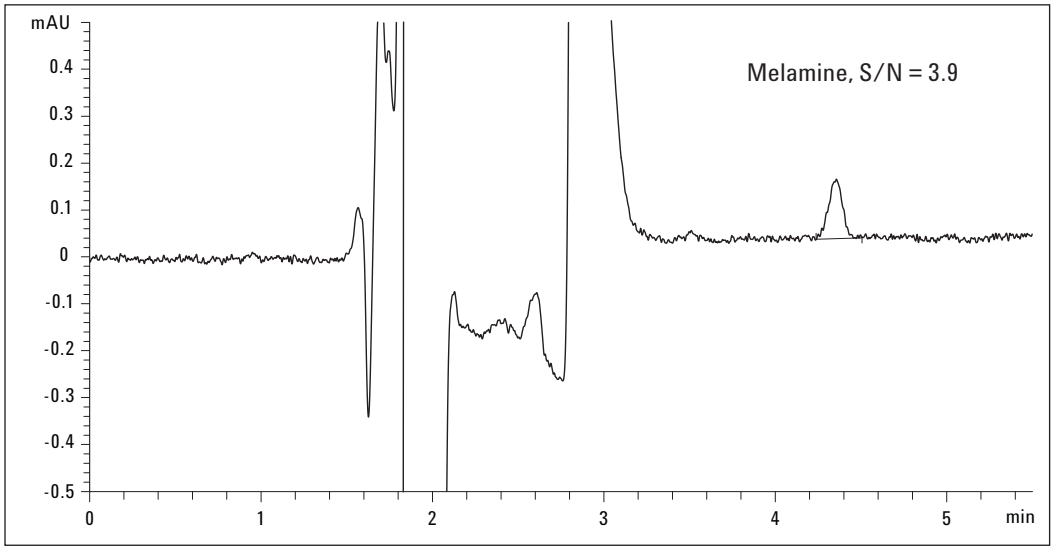


Figure 5
Limit of detection (LOD) for melamine at the concentration of 0.05 µg/mL.

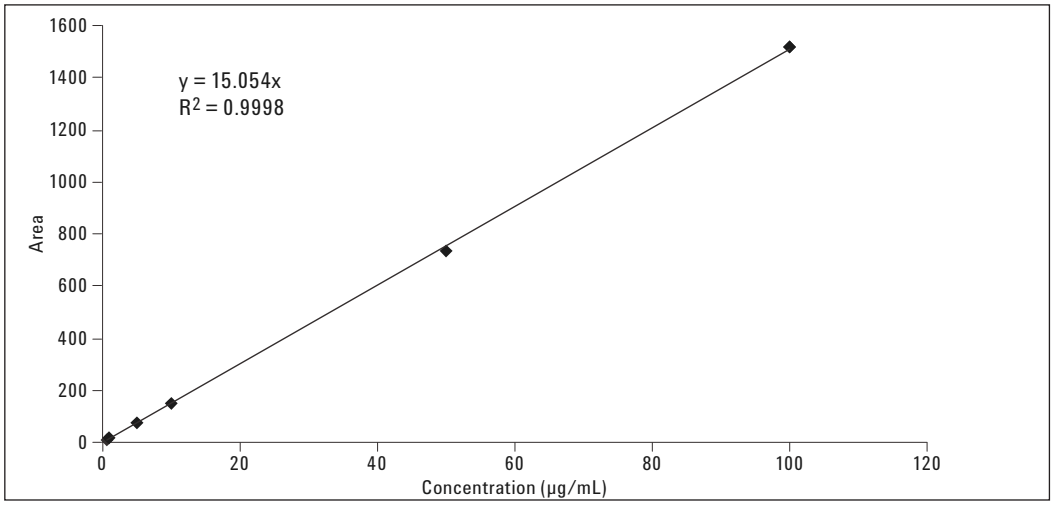


Figure 6
Calibration curve of IEC method.

Conclusions

Three approaches are described in this application note; the first is a reversed-phase ion-pair LC method employing Agilent 1120 compact LC or 1200 HPLC with an SB-C8 column. The second is a high-throughput method, which reduces the LC run time from 20 minutes to 6 minutes using the Agilent 1200 RRLC with the ZORBAX RRHT SB-C8 column. The last is an IEC method using the Agilent ZORBAX 300SCX column. Each successfully separates melamine from matrix interferences and provides identification by retention time and quantitative results. The results of this study, including sample cleanup with SampliQ SCX SPE cartridges and the three separation approaches, show that a complete solution from Agilent for the determination of melamine in dairy products is provided. The reversed-phase ion-pair method is based on the FDA and China national standards. However, the IEC method is simple, quick, sensitive, and robust. With this method, melamine can be successfully retained using a simple mobile phase without the presence of an ion-pair reagent.

References

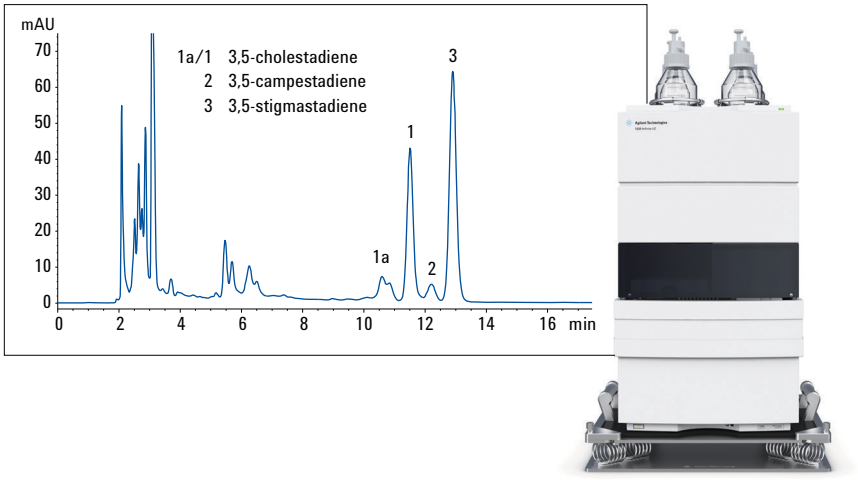
1. Updated FCC Developmental Melamine Quantitation (HPLC-UV), April 2, 2007
2. GB/T 22388-2008 Determination of melamine in raw milk and dairy products, October 7, 2008

Quality analysis of virgin olive oils – Part 1

Thermal treatment analysis – determination of 3,5-stigmastadienes in olive oil using the Agilent Infinity 1220 LC System with Diode Array Detector

Application Note

Food Analysis & Agriculture



Author

Sonja Schneider
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

3,5-stigmastadienes were analyzed in seven olive oil samples using the Agilent 1220 Infinity Mobile LC Solution to differentiate virgin from refined or other thermally-treated olive oil. No 3,5-stigmastadienes were detected in all of the tested virgin oils, in contrast to the partly refined olive oil sample, where a significant amount of 3,5-stigmastadienes was found. The analysis showed excellent linearity coupled with low limits of detection and limits of quantification. The analysis time could be shortened down to 5 minutes using a 50-mm, sub-2 µm column.

Due to the robust and rugged 1220 Infinity Mobile LC Solution, it is possible to perform olive oil analysis on-site as a starting point for quality analysis of virgin olive oils.

Introduction

Virgin olive oil can be created only by mild, cold pressing of the olives (*Olea europea* L.). Thermal or chemical treatment is not allowed in the procedure. After pressing, the virgin olive oils are only purified and filtered. The name, virgin olive oils, is only given to those oils produced by physical techniques such as pressing, filtration, decantation, and centrifugation (Regulation (EG) Nr. 1234/2007, Appendix XVI).

Refining crude vegetable oils removes impurities (for example, pigments, odors, flavoring, or bitter substances), which can have negative effects on the quality of the oils. Refining can improve flavor, stability, aroma, and color. However, during refining processes, many secondary metabolites are also removed or chemically altered.

There are different analytical methods to differentiate virgin from refined or thermally-treated olive oils. In addition to the determination of stigmastadienes and chlorophyll degradation products[1], the analysis of the concentration of polymerized triacylglycerides in olive oil is another important factor[2]. The quality of olive oils (especially regarding nutritive value) can be described in the amount of tocopherols, squalen, and fatty acid composition. Additional thermal treatment analysis and analysis regarding nutritive benefits will be addressed in upcoming Application Notes.

Virgin olive oils, obtained by cold pressing, do not contain measurable amounts of 3,5-stigmastadiene, less than 0.01 mg/kg[3]. Due to high temperatures in the bleaching and deodorizing part of the refining process, 3,5-stigmastadienes are formed by the dehydration of β-sitosterol (Figure 1), which is the most abundant steradiene found in vegetable oils[4].

The amount of stigmastadienes in commercially refined vegetable oils is dependent on the conditions applied during the refining process[4]. The determination of stigmastadienes in olive oils also detects minor amounts of refined oils in virgin olive oils and is, therefore, an important quality characteristic for virgin olive oils. Commercially refined vegetable oils normally contain a steradiene level between 1 and 100 mg/kg. The amount of 3,5-stigmastadiene in refined olive oils ranges between 0.3 and 0.9 mg/kg[4].

There are two major analytical methods for steradiene analysis in vegetable oils. The AOCS Official Method Cd26-96 (1990) and the IUPAC method[4]. Both methods describe sample preparation with saponification of the triacylglycerols and extraction using silica gel solid phase extraction (SPE) with subsequent gas chromatographic separation. However, the gas chromatographic separation runs the risk of interferences with other hydrocarbons. Another method for steradiene analysis is reversed phase HPLC according to EN ISO 15788-3:2004 (D) and Fiebig (1999)[5], which was used in this Application Note.

The 1220 Infinity Mobile LC Solution is a robust and rugged system for on-site measurement. It is resistant against shocks or vibrations during transportation in a mobile van. Due to the UV detection of the stigmastadienes analysis method, the 1220 Infinity Mobile LC Solution can be used in a mobile laboratory as a starting point for olive oil quality analysis before further quality analyses are applied in a stationary lab.

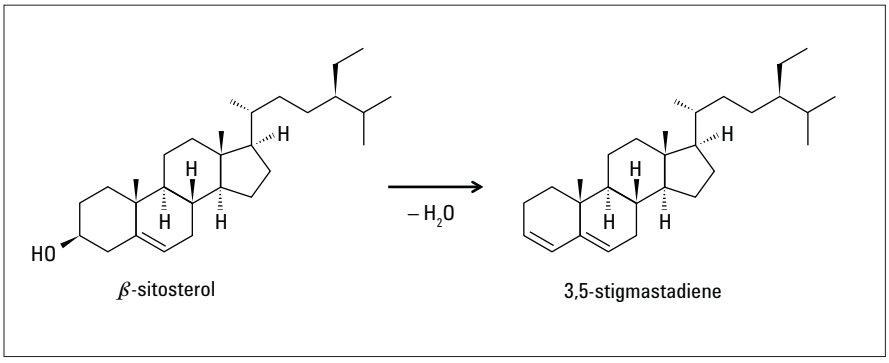


Figure 1
Formation of β-sitosterol to 3,5-stigmastadiene by dehydration.

Experimental

The Agilent 1220 Infinity Gradient LC system with a DAD (G4294B) was equipped with a dual gradient pump with integrated degasser, autosampler, column compartment, and the diode array detector. For transportation, the LC can be mounted on a transportation plate, 1220 Infinity Mobile Upgrade Kit (G4292A).

Sample

The internal standard 3,5-cholestadiene was purchased from Sigma-Aldrich, St. Louis, MO, USA and dissolved in 50% ACN and 50% methyl tert-butyl ether. Several olive oils (virgin and partly refined olive oil) were purchased in local stores. The SPE extraction was carried out using Agilent Bond Elut SI cartridges 5 g, 20 mL (p/n 14256026). Sample preparation was carried out according to EN ISO 15788-3:2004 (D) using the internal standard method.

Solvents

Acetonitrile (ACN), petroleum ether, and methyl tert-butyl ether were LC grade and purchased from Sigma-Aldrich, St. Louis, MO, USA.

Columns

Agilent LiChrospher C18, 4 × 250 mm, 5 µm (p/n 79925OD-584), Agilent ZORBAX Extend-C18 RRHT, 4.6 × 50 mm 1.8 µm (p/n 727975-902)

Software

- OpenLAB CDS ChemStation Edition for LC & LC MS Systems, Rev. C.01.04 [35]
- OpenLAB CDS 3D UV Add-On software.

Results and Discussion

The injected 3,5-cholestadien standard (10 µg/mL) showed the following chromatogram, (Figure 2) comprising two peaks for 3,5-cholestadien standard (1a and 1).

Chromatographic conditions

	Long run	Short run
Mobile phase:	ACN/methyl tert-butyl ether (70:30)	
Flow rate:	1 mL/min	
Isocratic run:	Stop time – 30 minutes	Stop time – 5 minutes
Injection volume:	10–50 µL	20 µL
Temperature TCC:	RT	
DAD:	235 nm/4 nm Ref.: off	
Peak width:	>0.05 minutes (1.0 seconds response time) (5 Hz)	

Table 1
Chromatographic conditions.

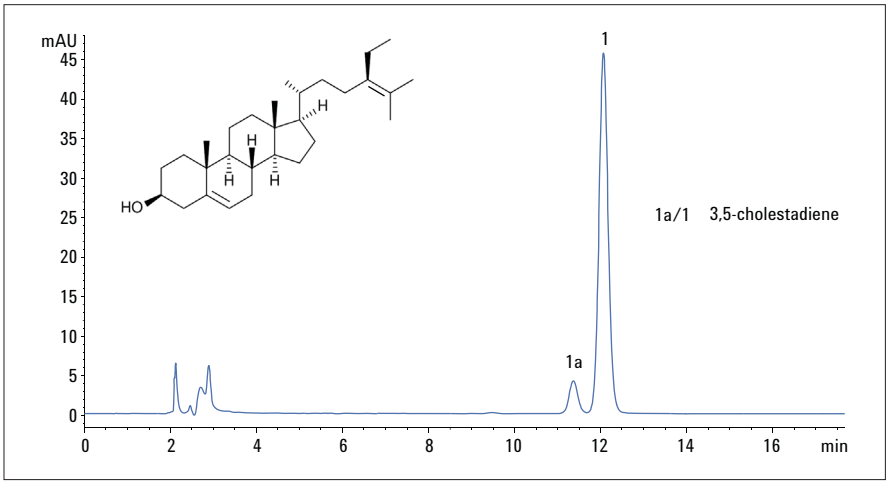


Figure 2
3,5-cholestadien standard solution separated on an Agilent LiChrospher C18, 4 × 250 mm, 5 µm.

Using a 3,5-cholestadien standard as an internal standard, the extracted sample from six different virgin olive oils revealed no additional peaks after the 3,5-cholestadien standard peaks (Figure 3). As expected, no 3,5-stigmastadienes were detected.

In contrast to virgin olive oils, 3,5-stigmastadienes were detected in partly refined olive oil (mix of refined and virgin oils), (Figure 4). The area precision for 3,5-cholestadien and 3,5-stigmastadienes was below 2.5 % for six consecutive runs of the partly refined olive oil sample.

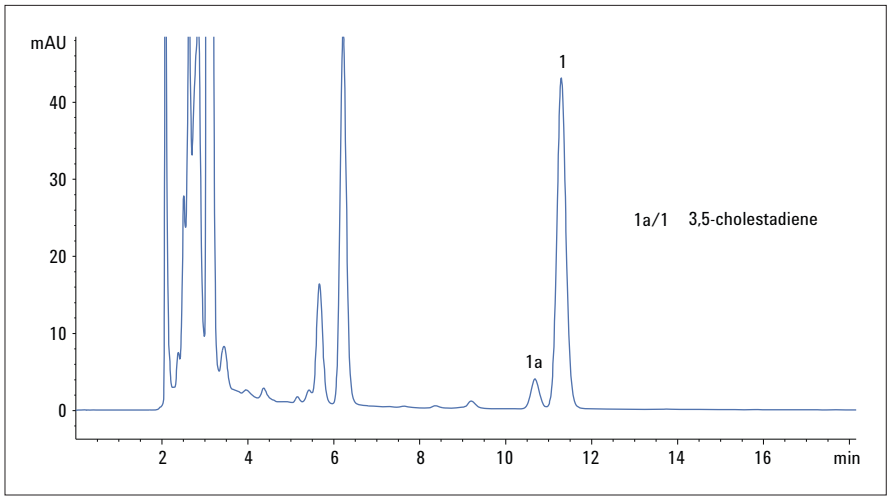


Figure 3
Virgin olive oil using the internal standard method of EN ISO 15788-3:2004 (D). No stigmastadienes were detectable.

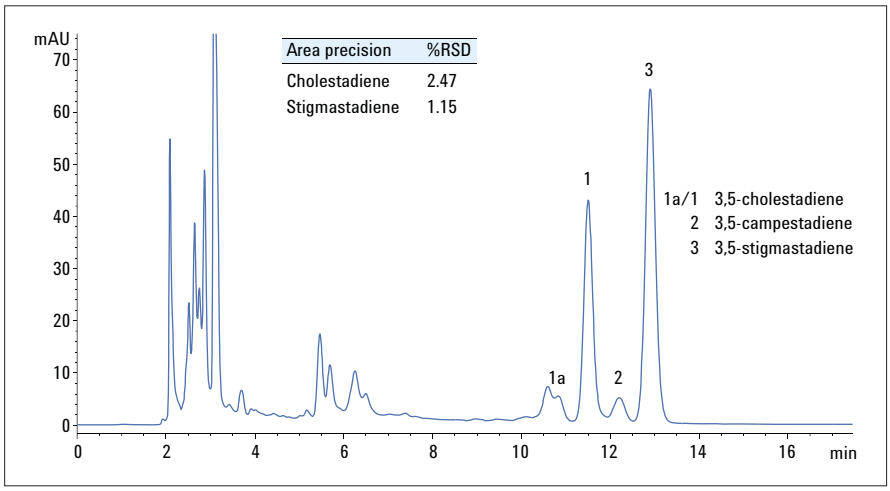


Figure 4
Detection of 3,5-stigmastadienes in partly refined olive oil.

Table 2 shows an overview for six virgin and one partly refined olive oil sample. No 3,5-stigmastadienes were detected in any of the tested virgin olive oils. The partly refined olive oil sample contained 0.63 mg 3,5-stigmastadienes per kg sample. The amount of 3,5-stigmastadienes was calculated using Formula 1 according to EN ISO 15788-3:2004 (D).

Linearity was determined using a dilution series of 3,5-cholestadiene ranging from 0.51 to 10,000 pg on column. Excellent linearity was found with a correlation factor of 0.99998.

In addition, the response factors confirmed the results except for the lowest concentration of 0.51 pg on column (data not shown). The response factors from 1.52 to 10,000 were within the ±5% range, representing excellent linearity.

The LOD and LOQ were evaluated from the concentration of 3,5-cholestadiene required to give at least a signal-to-noise ratio of 3 and 10, respectively. Table 3 displays LOD and LOQ for all for 3,5-cholestadiene, which are in the required range limit in virgin olive oils.

Olive oil	1	2	3	4	5	6	Mix of refined and virgin oils
Content of 3,5-stigmastadiene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.63 mg/kg

Table 2
Content of 3,5-stigmastadiene in different virgin and partly refined olive oil samples (n.d. not detectable).

$$w = \frac{A'_s \times M'}{A'_c \times m'}$$

w = Content of 3,5-stigmastadienes in mg per kg sample
A'_s = Peak area of 3,5-stigmastadienes
M' = Amount of injected internal standard in µg
A'_c = Peak area of 3,5-cholestadiene (both peaks)
m = Sample amount in g

Formula 1
Calculation of 3,5-stigmastadienes in mg per kg sample.

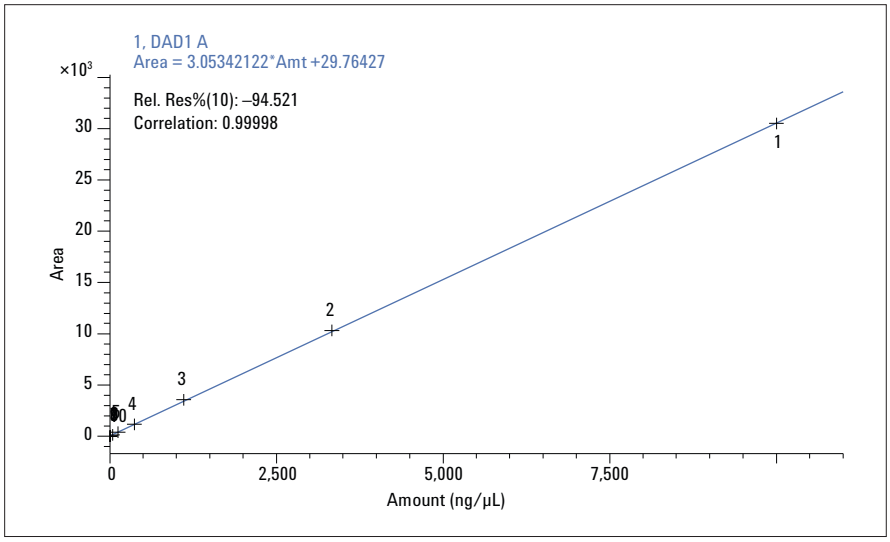


Figure 5
Linearity of 3,5-cholestadiene standard.

LOD	LOQ
226 pg on column	754 pg on column
4.52 µg/kg oil	15.1 µg/kg oil

Table 3
LOD and LOQ of 3,5-cholestadiene.

To accelerate analysis time, the run was shortened to 5 minutes using a 50-mm, sub-2 µm column (Agilent ZORBAX Extend-C18 RRHT, 4.6 × 50 mm 1.8 µm), still obtaining good resolution of the analytes in partly refined olive oil (Figure 6).

Summary and Conclusion

Seven olive oils were analyzed for 3,5-stigmastadiene to determine refining processes or other thermal treatments according to EN ISO 15788-3:2004 (D). As expected, no 3,5-stigmastadienes were detected in any of the tested virgin oils. In contrast, in partly refined olive oil, a sample containing refined and virgin oils, the amount of 3,5-stigmastadienes found was 0.63 mg per kg sample. To determine linearity and LOD/LOQ, the internal standard 3,5-cholestadiene was diluted from 10,000 to 0.51 pg injected amount. Excellent linearity was found from 1.52 pg to 10,000 pg, confirmed by a correlation factor of 0.99998, and response factors in a ±5% range from the average value, enabling highly accurate quantification. LOD and LOQ were found in the low µg/kg oil sample. The analysis time could be shortened to 5 minutes using a 50-mm, sub-2 micron column.

Using the Agilent 1220 Infinity Mobile LC Solution for the analysis of 3,5-stigmastadienes in olive oils, it is possible to perform olive oil analysis on-site. The 1220 Infinity Mobile LC Solution is a robust and rugged system that can be used in a mobile laboratory. The analysis of 3,5-stigmastadienes can act as a starting point for olive oil quality analysis on-site to differentiate virgin from refined olive oils. Due to the low LOD and LOQ, even small amounts of refined oil can be detected with this application. Based on those measurements, further quality analysis can then be applied in a stationary laboratory.

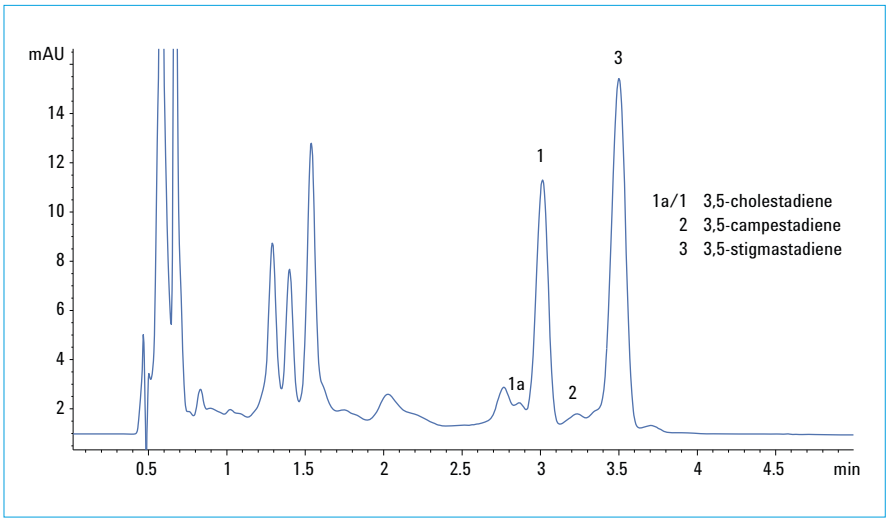


Figure 6
Short analysis of partly refined olive oil using an Agilent ZORBAX Extend-C18 RRHT, 4.6 × 50 mm 1.8 µm.

References

1.
Quality Analysis of Virgin Olive Oils – Part 3, Agilent Application Note, Publication Number 5991-1896EN, **2013**.

2.
Quality Analysis of Virgin Olive Oils – Part 2, Agilent Application Note, Publication Number 5991-1895EN, **2013**.

3.
Dobarganes *et al.*, The determination of stigmastadienes in vegetable oils (technical report), *Pure & Appl. Chem.,* Vol. 71, No 2, pp. 349-359, **1999**.

4.
L Brühl and HJ Fiebig. Qualitätsmerkmale kaltgepresster Speiseöle, *Fat. Sci., Technol. 97. Jahrgang Nr. 6,* **1995**.

5.
HJ Fiebig. Determination of β-3,5-steradienes in fats and oils, *Fett/Lipid,* 101, 442-445, **1999**.

Pharmaceutical Applications

Throughout all stages of the pharmaceutical value chain, from early drug discovery to launching successful products, there is increasing pressure to obtain reliable results quickly. Specifically in pharmaceutical manufacturing the QA/QC laboratory must ensure that quality standards are constantly met to guarantee outstanding quality of raw materials, intermediates and final products. For herbal medicines and Traditional Chinese Medicines (TCM), it must also be assured that regulatory standards are met. Applications in this section show high reliability of data of the 1220 Infinity LC.

Analysis of amoxicillin and five impurities on the Agilent 1220 Infinity LC System

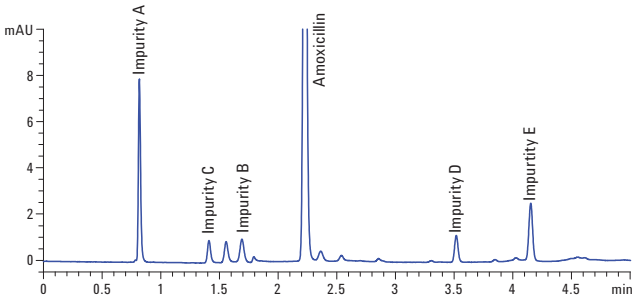
LC analysis of impurities down to the 0.01% level with long sub-2-µm columns, high flow rates and back pressure greater than 400 bar

Application Note

Drug Development and Pharmaceutical QA/QC

Authors

A. Koeberlein
Frankenthal, Germany
A.G. Huesgen
Agilent Technologies
Waldbronn, Germany



Abstract

Amoxicillin is an antibacterial drug widely used against gram-positive and gram-negative organisms. In pharmaceutical drug discovery and development, it is crucial to analyze impurities due to their potential for toxic effects on humans. Amoxicillin and its impurities were analyzed on the Agilent 1220 Infinity LC System on a 150 mm × 4.6 mm column with 1.8 µm particles. When using a flow rate of 2 mL/min (at 525 bar), the analysis was completed within seven minutes. Under these conditions, impurities down to a level of 0.01% could be detected. The Agilent 1220 Infinity LC System is an ideal instrument for impurity analysis in a pharmaceutical quality control laboratory.

Introduction

In pharmaceutical drug discovery and development, it is crucial to analyze for impurities due to their potential for toxic effects on humans. An impurity could be the active pharmaceutical substance itself, a minor byproduct from the production process, a secondary substance in a drug isolated from a natural source, a metabolite created in the human body, or a degradation product of the pharmaceutical agent created under storage conditions. Regulatory agencies stipulate that in the final drug, the amount of an impurity is below 0.01% of the main compound.

Recently, a number of publications have dealt with the analysis of amoxicillin and its impurities by HPLC with UV or MS detection.[1,2,3] Amoxicillin is an antibacterial drug widely used against gram-positive and gram-negative organisms. It can degrade to several different byproducts, as illustrated in the pathways in Figure 1. Amoxicillin is a polar substance, and its separation requires a high amount of water in the mobile phase.

In this Application Note, we use a gradient method in combination with UV detection for the determination of amoxicillin and related impurities. Precision of areas and retention times as well as limits of detection (LOD) and limits of quantitation (LOQ) for the impurities were evaluated. Linearity in the low nanogram range (trace level) was evaluated for impurities. The Agilent 1220 Infinity LC System is an ideal instrument for impurity analysis in a pharmaceutical quality control laboratory.

Experimental

Instrumentation

The instrument used in this application is the Agilent 1220 Infinity LC System in the following configuration: Gradient Pump, Autosampler, Column Oven and Variable Wavelength Detector.

Analyzing amoxicillin requires a column that can tolerate 100% water as a starting condition. The Agilent ZORBAX SB-Aq column, which is compatible with the highest percentages of water in the mobile phase was used for all experiments. The software used was ChemStation, revision B.04.02.

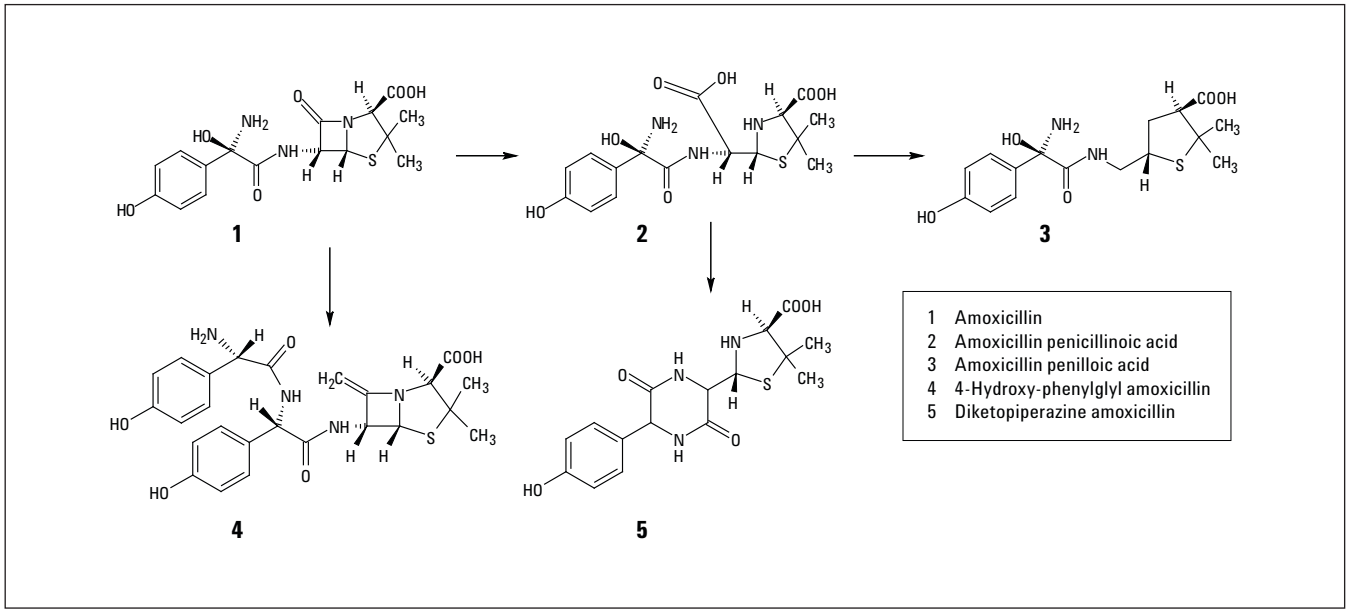


Figure 1
Degradation pathways of amoxicillin.

Chromatographic conditions

Sample:	Amoxicillin diluted in pure water
Column:	Agilent ZORBAX SB-Aq 4.6 mm × 150 mm, 1.8 µm p/n 829975-914
Mobile phase:	A: Water phosphate buffer (0.01 mol/L), pH=4.8 B: ACN

Gradient:

Time [min]	Component A [%]	Component B [%]
0	100	0
5	78	22
7	60	40

Flow rate:	2.0 mL/ min, max pressure 525 bar
Stop time:	7 min
Post time:	3 min
Detector:	VWD 229 nm
Peak width:	PW > 0.025 min, 20 Hz
Injection Vol:	1 µL
Column temp:	40 °C

Sample preparation

Sample compounds:

- Amoxicillin trihydrate
- Impurity A, p-hydroxy-Phenylglycine
- Impurity B, Amoxicillin Penicilloic acid
- Impurity C, 6-Aminopenicillanic acid
- Impurity D, p-hydroxy-Phenylglycyl Amoxicillin
- Impurity E, Diketopiperazine-Amoxicillin

A stock solution of amoxicillin was prepared in water with a concentration of 1 mg/mL. Solutions for the evaluation of impurity data were prepared in water (Table 1). Different concentration levels were used for testing the precision of retention times and areas for the evaluation of LOD, LOQ and linearity.

Level	1 Amount (ng/µL)	2 Amount (ng/µL)	3 Amount (ng/µL)	4 Amount (ng/µL)	5 Amount (ng/µL)	6 Amount (ng/µL)
Impurity A	6.9	3.45	1.38	0.69	0.345	0.138
Impurity B	6.96	3.48	1.392	0.696	0.348	0.1392
Impurity C	10.9	5.45	2.18	1.09	0.545	0.218
Impurity D	3.57	1.785	0.714	0.357	0.1785	0.0714
Impurity E	5.28	2.64	1.056	0.528	0.264	0.1056

Table 1
Concentratiion levels of impurities.

Results and discussion

Precision of retention times and areas

Precision of areas and retention times was evaluated with concentration level 1 (Figure 2). The injection volume was 1 µL. The resolution for impurity B is 1.97.

Other impurities and degradation products are observed in the chromatogram, but in Figure 2, only the impurities and degradation products shown in Table 1 were determined.

Table 2 shows the precision of retention times and areas of amoxicillin impurities.

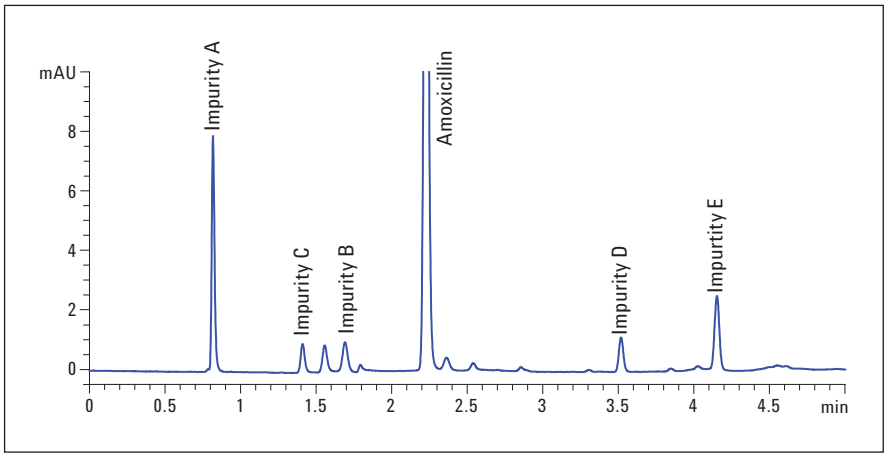


Figure 2
Concentration level 1 for testing precision of retention times and areas over eight runs, 1 µL injected, maximum pressure of 525 bar at 2 mL/min flow rate.

n=8	Impurity A Ret Time (min)	Impurity A Area (mAU's)	Impurity B Ret Time (min)	Impurity B Area (mAU's)	Impurity C Ret Time (min)	Impurity C Area (mAU's)	Amoxicillin Ret Time (min)	Amoxicillin Area (mAU's)	Impurity D Ret Time (min)	Impurity D Area (mAU's)	Impurity E Ret Time (min)	Impurity E Area (mAU's)
Mean:	0.818	10.670	1.404	1.884	1.686	2.373	2.224	60.416	3.515	2.487	4.152	5.675
S.D.:	2.890E-04	9.549E-02	3.330E-03	1.254E-02	2.560E-03	9.084E-02	2.240E-03	1.868E-01	7.860E-04	3.342E-02	4.220E-04	2.803E-02
RSD:	0.035	0.895	0.237	0.666	0.152	3.828	0.101	0.309	0.002	1.344	0.022	0.494

Table 2
Precision of RT and areas of amoxicillin and impurities for level 1 concentration.

The precision of retention times is between 0.002% and 0.24% RSD for all peaks.

The precision of areas is between 0.5% and 3.9% RSD for impurities with area counts between 1.9 and 11 mAU's.

Linearity

Linearity was tested over all six concentration levels (Figure 3). The linearity is better than 0.999 for the factor of correlation.

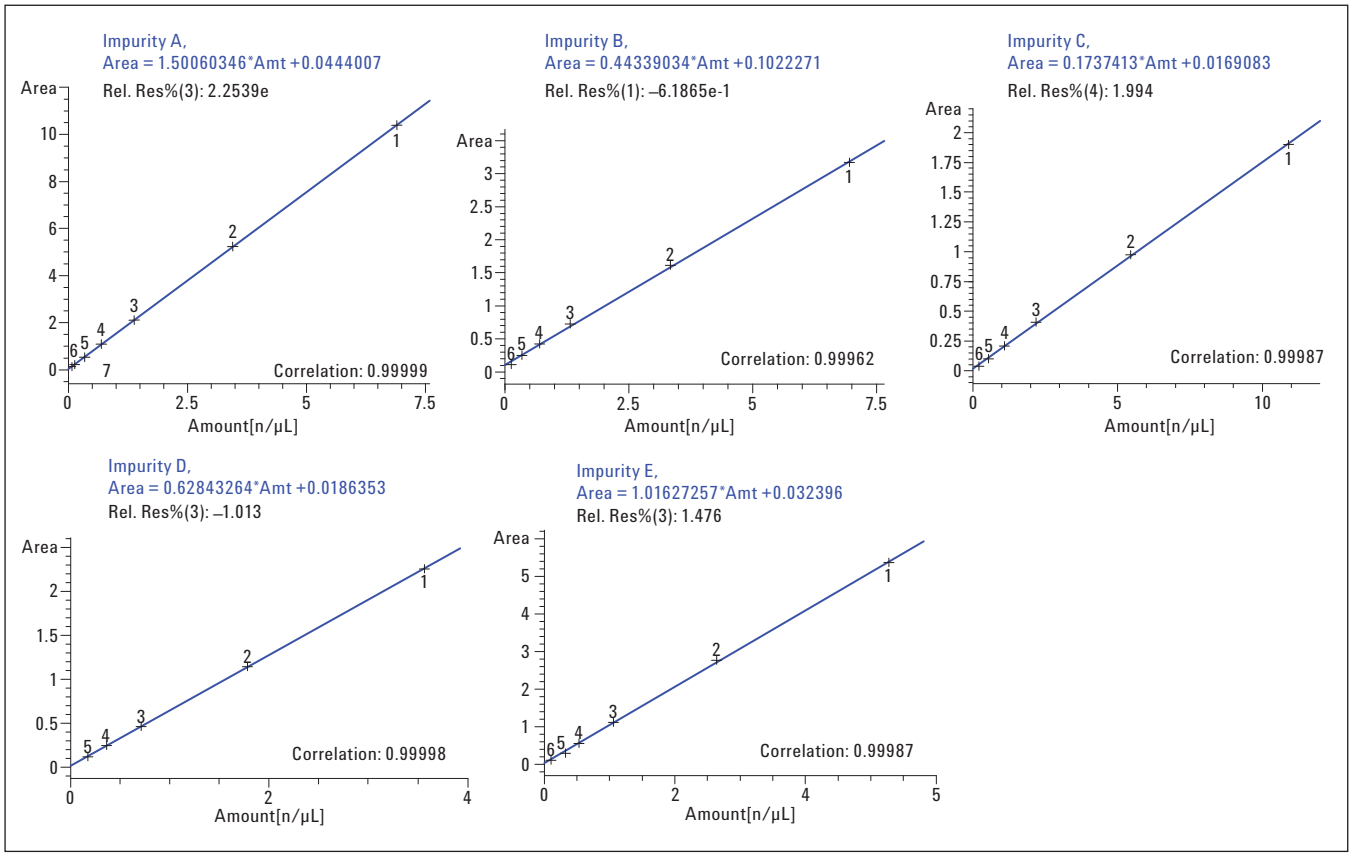


Figure 3
Linearity of impurities over all six concentration levels.

The limit of detection for the impurities was between 0.02 ng and 0.17 ng injected amount with a signal-to-noise ratio of 2 (Figure 4 red trace). Level 6 was used to determine the minimum detectable level. The limit of quantitation was 20 times higher, close to concentration level 4, (Figure 5 blue trace).

In Table 3, the results for LOD and LOQ are combined.

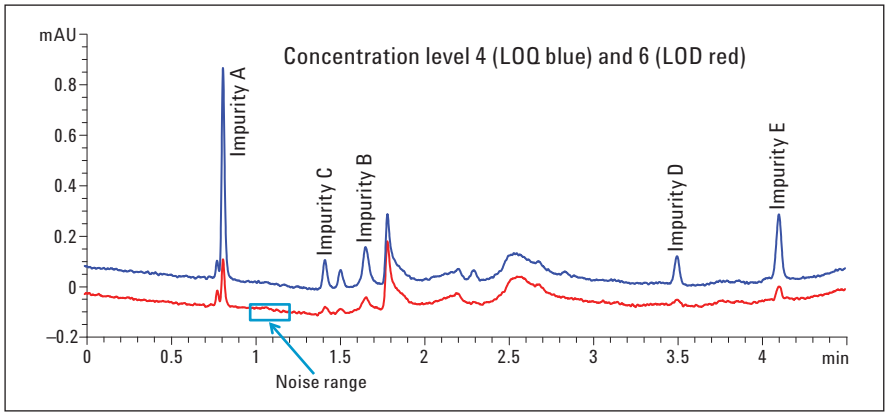


Figure 4
Concentration level 4 (blue trace) and level 6 (red trace), 1 µL injected.

	LOD amount (ng/µL)	Concentration level 6 amount (ng/µL)	LOQ (times 20 LOD) amount (ng/µL)	Concentration level 4 amount (ng/µL)
Impurity A	0.02	0.138	0.4	0.69
Impurity C	0.17	0.1392	3.4	0.696
Impurity B	0.06	0.218	1.2	1.09
Impurity D	0.075	0.0714	1.5	0.357
Impurity E	0.047	0.1056	0.94	0.528

Table 3
Results for LOD and LOQ.

Analysis of the amoxicillin sample

Figure 5 shows the chromatogram of the amoxicillin sample. The sample was dissolved in water to a final concentration of 1 mg/mL.

The results are presented in Table 4, showing that impurities in the 0.01% range can be detected. Amoxicillin is present at a percentage level of 96%. The percentage levels for the impurities are calculated in relation to this level.

Conclusion

Amoxicillin and its impurities were analyzed on the Agilent 1220 Infinity LC System using a 150 mm × 4.6 mm column with 1.8 µm particles. At a flow rate of 2 mL/min (at 525 bar) the analysis was completed within seven minutes. Under these conditions, impurities down to a level of 0.01% could be detected. This shows that the Agilent 1220 Infinity LC System is an ideal instrument for impurity analysis in a pharmaceutical quality control laboratory.

References

1. Ch.B.V.N.Raju, et al., "RP-HPLC method for analysis of related substances in Amoxicllin Drug substance," Acta Chromatographia 21, 1, 57–70, **2009**

2. Edgar Naegele et al., "Structure elucidation of degradation products of the antibiotic drug Amoxicillin," Agilent Technologies publication 5989-2470EN, **2005**

3. Edgar Naegele, "Statistic evaluation of mass accuracy measurements by ESI TOF with a sample of degradation products from the antibiotic drug Amoxicillin," Agilent Technologies publication 5989-3561EN, **2005**

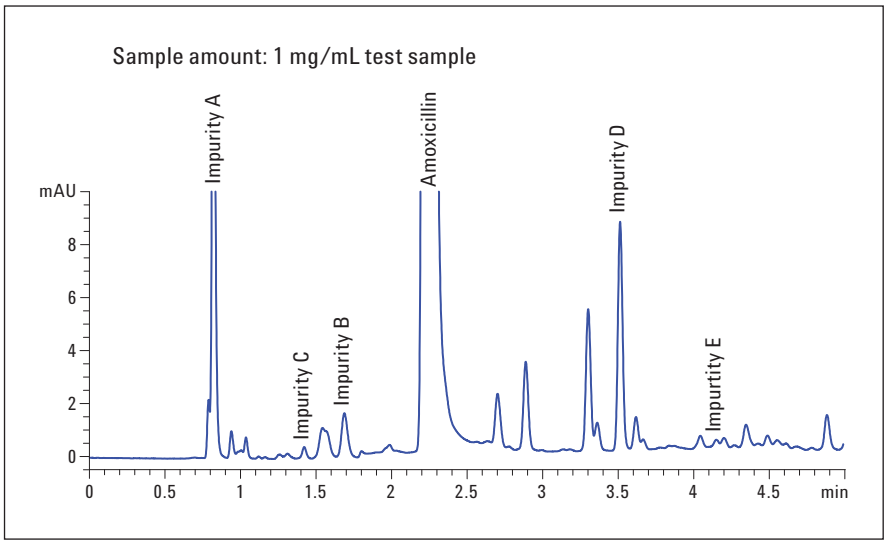


Figure 5
Amoxicillin sample, 1 µL injected.

	Area (mAU's)	ng/µL	Percentage
Impurity A	18.35269	12.20062	0.5
Impurity C	0.340461	1.86227	0.01
Impurity B	3.96829	8.71932	0.11
Impurity D	8.67657	13.77702	0.25
Impurity E	0.719148	0.675756	0.02
Amoxicillin	3360.63696		96

Table 4
Results of real life sample.

Analysis of Paracetamol and Aspirin in pain relievers on the Agilent 1220 Infinity Isocratic LC System with manual injector

Excellent chromatographic results at lowest costs

Application Note

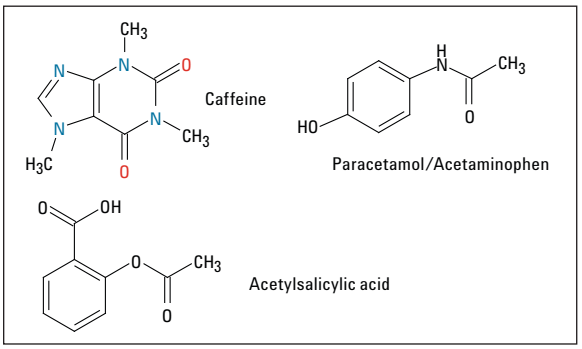
Pharmaceuticals

Author

A.G.Huesgen

Agilent Technologies

Waldbronn, Germany



Abstract

The Agilent 1220 Infinity LC System is an integrated LC system consisting of an isocratic pump, manual injector and variable wavelength detector. This Application Note describes the analysis of paracetamol and aspirin to demonstrate that typical acceptance criteria for a USP method can be fulfilled. These criteria are:

- Precision of areas < 2% RSD
- Precision of retention times < 0.5% RSD
- Resolution > 1.4
- Tailing factor < 1.2

The data from this analysis show that the Agilent 1220 Infinity LC System provides excellent performance at low costs.

Introduction

The Agilent 1220 Infinity LC System is a liquid chromatography (LC) system for routine standard analysis. Due to its extraordinary pressure range up to 600 bar, the system can perform UHPLC applications. It is an easy to use, integrated LC system consisting of an isocratic pump, manual injector and variable wavelength detector (VWD).
The isocratic pump has a flow range of 0.2 to 10 mL/min (5 mL at 600 bar, 10 mL at 200 bar) and an integrated degasser. The VWD detector features 80 Hz data acquisition rate and a wavelength range from 190 nm to 600 nm.
The system can be upgraded according to growing needs with:

- Oven upgrade kit – adds a click-in oven to your Agilent 1220 Infinity LC System
- Isocratic to gradient pump upgrade kit – adds gradient capabilities to your isocratic Agilent 1220 Infinity LC System
- Manual injector to autosampler upgrade kit – exchanges your manual injector with an autosampler

Paracetamol and aspirin were chosen as examples in this analysis to demonstrate that typical acceptance criteria for a USP method can be fulfilled (Fgure 1). The isocratic USP method with UV detection according to USP/NF 23 was applied for analysis.

Experimental

Instrumentation

For the analysis of paracetamol, aspirin and caffeine, an Agilent 1220 Infinity LC System with the following configuration was used:

- Agilent 1220 Infinity LC System (G4286B) consisting of an isocratic pump, manual injector and VWD
- 20 µL loop (p/n 0100-1922) installed

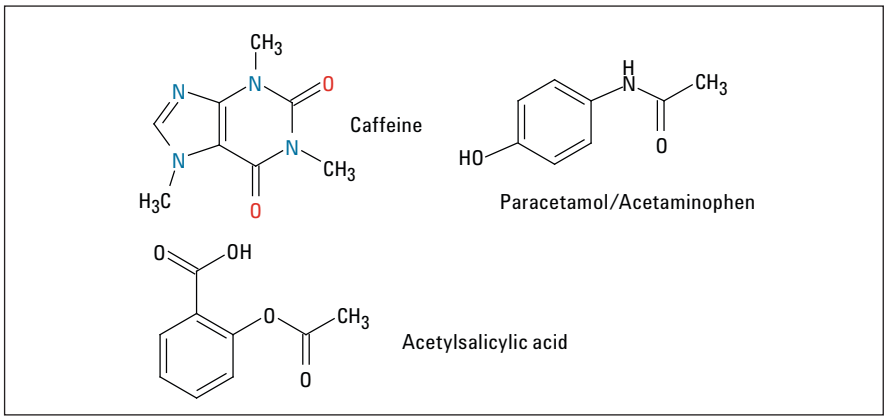


Figure 1
Structures of acetaminophen (paracetamol), aspirin and caffeine.

Chromatographic conditions according to USP method	
Column:	Agilent ZORBAX Eclipse Plus C18 Column, 3 mm × 100 mm, 3.5 µm (internal diameter 35% less than original method, particle size 30% less than original method)
Mobile phase:	Water/methanol/acetic acid = 69/28/3
Pump settings:	No gradient (in accordance with EP regulations)
Stop time:	6 min
Flow rate:	1 mL/min, isocratic (50% less than original method)
Injection volume:	20 µL
Column temp:	Ambient (Laboratory temperature between 24 °C and 25 °C)
Detector:	Agilent 1220 Infinity LC System with 10 mm path length flow cell Peak width 0.05 min (10 Hz) Signal 275 nm

The original method was changed according to the typically allowed changes for chromatographic parameters (Table 1).

Chromatographic parameter	Typically allowed changes
Mobile phase pH	± 0.2 units
Concentration of salts in buffers	± 10%
Ratio of mobile phase percentages	± 30% of the minor component, or 0.2% absolute of that component, whichever is greater. However a change in any component cannot exeed ± 10% absolute, nor can the final concentration be reduced to zero
Wavelength of UV detector	No change permitted
Column length	± 70%
Internal diameter of column	± 50%
Particle size of column packing material	Can be reduced by 50%
Flow rate	± 50%
Injection volume	Increased up to twice the volume specified, provided no adverse effects. Must be within stated linearity range of the method
Column compartment temperature	± 10°C

Table 1
Typically accepted changes for USP methods.

Preparation of samples

The reference solution was prepared according to the concentrations listed in Table 2.

Results and discussion

System suitability testing was performed to verify that the LC system fulfills the acceptance criteria typical for USP methods. The following acceptance criteria had to be fulfilled:

- Precision of areas must be < 2% RSD
- Precision of retention times must be < 0.5% RSD
- Resolution must be > 1.4 for benzoic acid
- Tailing factor < 1.2

An overlay of six consecutive chromatograms is shown in Figure 2. Table 3 combines the results to show that the acceptance criteria are fulfilled.

Conclusion

The Agilent 1220 Infinity LC system is a liquid chromatography (LC) system for routine standard analysis. Due to its extraordinary pressure range up to 600 bar, the system can also perform UHPLC applications. It is an integrated LC system consisting of an isocratic pump, manual injector and variable wavelength detector (VWD). The application example of the analysis of pain relievers shows that typical USP acceptance criteria are fulfilled on the Agilent 1220 Infinity LC system, showing excellent performance at lowest costs.

	Stock solution in mobile phase	1:5 diluted in water
Acetaminophen	5.5 mg/10 mL	1.1 µg/10 µL
Caffeine	1.3 mg/10 mL	0.26 µg/10 µL
Aspirin	3.9 mg/10 mL	0.78 µg/10 µL
Benzoic acid	4 mg/10 mL	0.8 µg/10 µL
Salicylic acid	4 mg/10 mL	0.8 µg/10 µL

Table 2
Sample concentration.

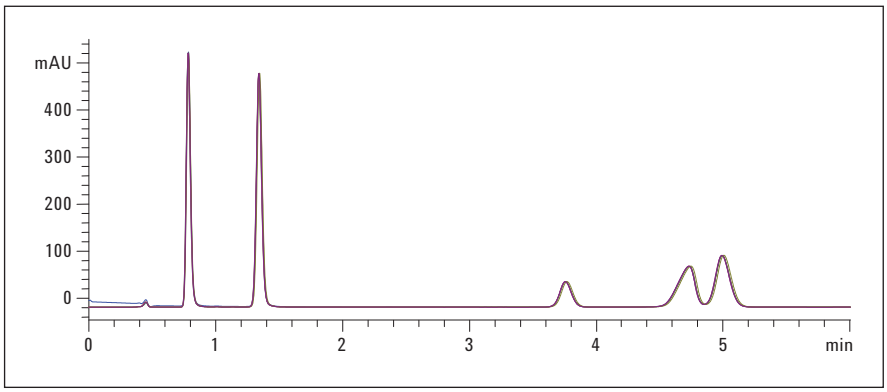


Figure 2
Overlay of chromatograms, six consecutive runs injected with manual loop injector.

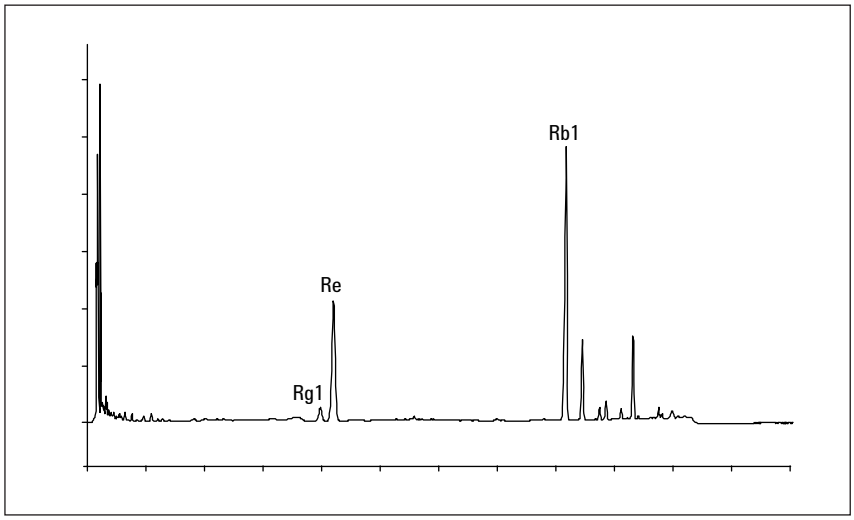
Compound	Retention time (min) Average	RSD RT (%)	RSD area (%)	Resolution (hH)	Peak tailing
Acetaminophen	0.784	0.208	0.371		1.179
Caffeine	1.341	0.209	0.341	8.117	1.105
Aspirin	3.756	0.147	0.100	19.557	1.041
Benzoic acid	4.730	0.129	0.195	4.600	0.800
Salicylic acid	4.991	0.117	0.191	1.139	1.005

Table 3
Results for retention time and area precision, resolution data and peak tailing.

Analysis of ginseng and American ginseng using the Agilent 1120 Compact LC

Application Note

Zhixiu Xu



Abstract

The Agilent 1120 Compact LC is the system of choice for conventional, analytical scale liquid chromatography. It is an intergrated LC designed for ease of use, performance and reliability. It is ideally suited for the routine analysis of Traditional Chinese Medicines (TCMs) on account of its capability to achieve highly precise retention times and peak areas, and low detection limits for the analyzed compounds. This Application Note shows the chromatograms obtained with optimizd methods for the most well-known TCMs ginseng and American ginseng, which show different peak profiles and different concentrations of certain saponins.

5989-7458EN

Introduction

Traditional Chinese Medicines (TCMs) have a long history of use and their therapeutic effects are well known in China and other countries. Ginseng, perhaps the most well-known TCM, has long been used as a tonic, anti-fatigue, sedative and anti-gastric ulcer drug. It is also widely used in different TCM preparations. Another well-known TCM, American ginseng, has similar therapeutic effects as ginseng but there are also some differences because of the different saponin contents.

According to the method in the pharmacopeia of the People's Republic of China[1], ginseng must be analyzed by HPLC to determine the ginsenosides Rg1, Re and Rb1. Similar requirements exist for the determination of these ginsenosides in American ginseng. These requirements make the determination of the ginsenosides Rg1, Re and Rb1 in ginseng and American ginseng important for quality control of TCM raw materials and final preparations.

In this study an HPLC analysis method was developed using the Agilent 1120 Compact LC for the determiniation of ginsenosides in ginseng and American ginseng.

Experimental

Equipment

- Agilent 1120 Compact LC comprising gradient pump with integrated degasser, autosampler with vial tray, column oven and variable wavelength detector, see figure 1
- Agilent HC-C18(2), high carbon load, 150 x 4.6 mm, 5 µm particle size column
- Agilent EZChrom Elite Compact software

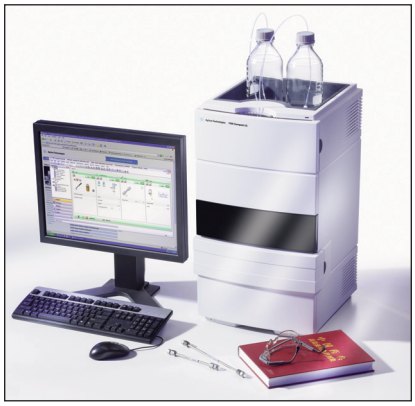


Figure 1
Agilent 1120 Compact LC

Samples and sample preparation

Ginseng and American ginseng were purchased from a local TCM store and samples prepared as follows. 1 g of powder was weighed and dissolved in 50 mL of water saturated n-butanol. The solution was treated ultrasonically for 30 minutes and centrifuged for 5 minutes at 300 rpm. The solvent was evaporated and the residue dissolved in 5 mL methanol. The final solution was filtered through a 0.20 µm membrane before injection.

Chromatographic conditions

- Mobile phase:
  A: Water, B: ACN
- Gradient: 0 min, 19 %B;
  35 min, 19 %B; 55 min, 29 %B;
  70 min, 29 %B; 100 min, 40 %B
- Flow rate: 1.0 mL/min
- Injection volume: 10 µL
- Column temperature: 40 °C
- Detection wavelength: 203 nm

Results and discussion

The chromatogram of the ginseng separation is shown in figure 2. The method used to obtain this chromatogram was the same as the method specified in the pharmacopeia of People's Republic of China. The chromatogram shows excellent separation of all the target compounds. The ginsenosides Rg1 and Re are well separated, demonstrating that the Agilent 1120 Compact LC is well suited for this analysis.

The chromatogram of the American ginseng separation is shown in figure 3. The chromatogram shows excellent separation of the target ginsenosides.

From the chromatograms of ginseng and American ginseng, it can be seen that the profiles of the two samples as well as the concentrations of the ginsenosides are different. For complex TCM samples such as ginseng and American ginseng, the Agilent 1120 Compact LC is a reliable tool to obtain good results in routine analysis work.

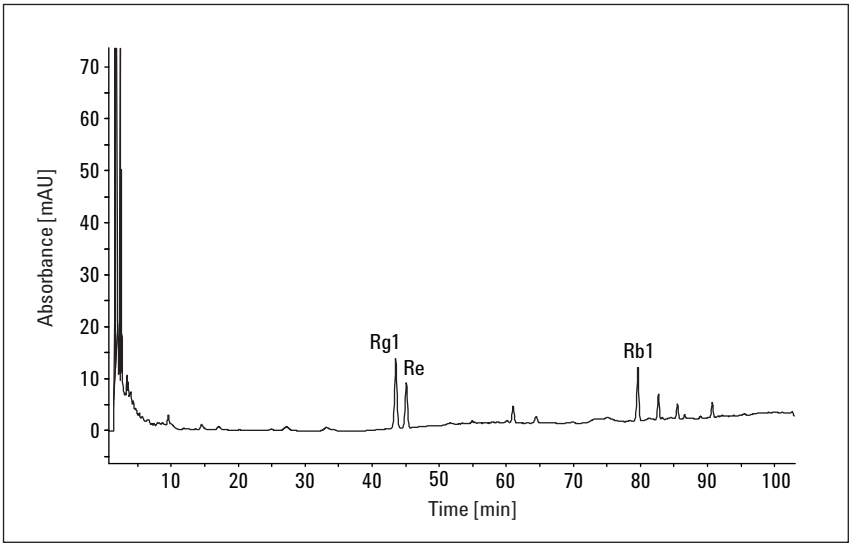


Figure 2
Chromatogram of ginseng analyzed with the CHP method on an Agilent 1120 Compact LC system.

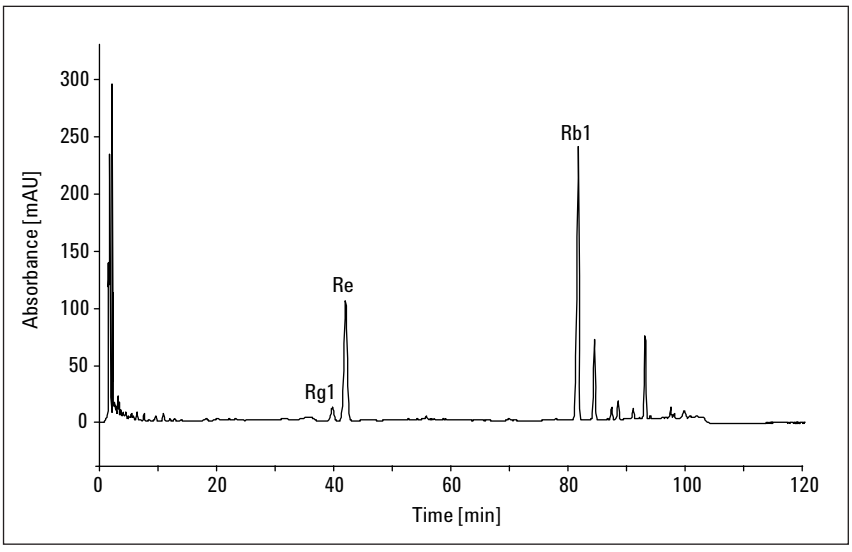


Figure 3
Chromatogram of American ginseng analyzed on an Agilent 1120 Compact LC system.

Conclusion

Although Traditional Chinese Medicines are complex natural products, this study demonstrated that the Agilent 1120 Compact LC was capable of analyzing the active components and achieving excellent separation performance. The results proved that the Agilent 1120 Compact LC is ideal for routine quality control testing of complex TCM samples.

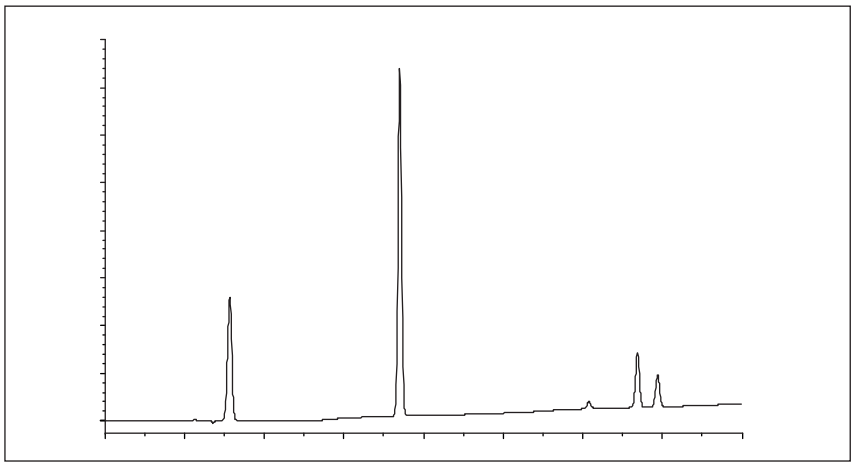
Reference

1.
Pharmacopeia of the People's Republic of China, Volume I, **2005**.

Development and validation of a method for simultaneous determination of paracetamol, diclofenac and ibuprofen using the Agilent 1120 Compact LC

Application Note

Angelika Gratzfeld-Huesgen
Patrik Hoerth
Daniel Thielsch



Abstract

The Agilent 1120 Compact LC is the system of choice for conventional, analytical scale liquid chromatography. It is an intergrated LC designed for ease of use, performance and reliability. It is ideally suited for the analysis of pharmaceuticals on account of its capability to achieve highly precise retention times and peak areas, and low detection limits for the analyzed compounds. In this Application Note, data is presented that demonstrates:

- Excellent retention time precision < 0.07 % RSD
- Excellent area precision < 1.0 % RSD for baseline separated peaks
- Excellent linearity with coefficient of correlation > 0.9999
- Limit of detection (LOD) 13 – 298 pg for all compounds analyzed

5989-7457EN

Introduction

For the routine analysis of pharmaceuticals compounds and impurities in QA/QC it is important to use LC systems that are highly accurate, precise and robust. The Agilent 1120 Compact LC is based on a proven robust design and delivers the required quality of data. This makes it ideally suited for routine QA/QC analysis of pharmaceutical compounds. In this study, the precision, linearity and limits of detection (LOD) of several pharmaceutical compounds were evalutated.

Experimental

Equipment

- Agilent 1120 Compact LC comprising gradient pump with integrated degasser, autosampler with vial tray, column oven and variable wavelength detector, see figure 1
- Agilent TC-C18(2), high carbon load, 150 x 4.6 mm, 5 µm particle size column
- Agilent EZChrom Elite Compact software

Chromatographic conditions

- Mobile phase:
  A: Water + 0.05 % TFA
  B: ACN + 0.045 % TFA
- Gradient:
  0 min, 25 %B;
  1 min, 25 %B;
  4 min, 60 %B;
  7 min, 70 %B
- Flow rate: 1.5 mL/min
- Injection volume: 3 µL
- Column temperature: 40 °C
- Detection wavelength: 230 nm
  Peakwidth: > 0.05 min
- Run time: 8 min
- Post time: 2 min

Results and discussion

Paracetamol, ibuprofen and diclofenac were chosen as example compounds for the evaluation of precision, linearity and LOD. Benzoic acid was used as internal standard (ISTD). Table 1 lists the concentration levels used.
The chromatographic method was set up so that all compounds were baseline separated. Paracetamol posed a particular problem because it elutes from the C-18 phase column with almost no retention. The start conditions were therefore selected so that paracetamol showed at least some retention on the selected column. Further, the objective of method development was to keep the total analysis

Compound	Ibuprofen ng/3 µL	Benzoic acid ng/3 µL	Paracetamol ng/3 µL	Diclofenac ng/3 µL
Level 1	38.15	71.25	56.7	25.2
Level 2	19.075	36.625	28.35	12.6
Level 3	9.538	18.313	14.175	6.3
Level 4	4.769	9.156	7.088	3.15
Level 5	2.384	4.578	3.544	1.575
Level 6	1.192	2.289	1.772	0.788
Level 7	0.596	1.145	0.886	0.394

Table 1
Drug compounds and concentration levels.

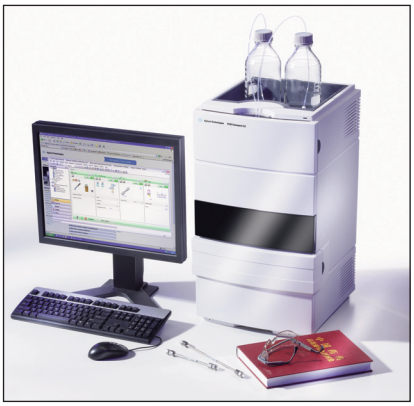


Figure 1
Agilent 1120 Compact LC

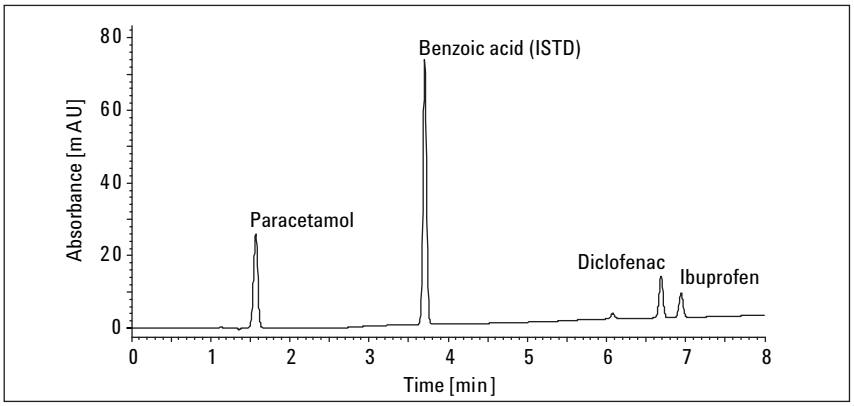


Figure 2
Analysis of pharmaceutical drugs, showing excellent resolution.

time as short as possible. The run and equilibration time could be limited to 10 minutes (figure 2). The mobile phase contained trifluoric acetic acid as modifier. This influenced positively both retention and peak shape. The first gradient slope at 4 minutes was fast enough to keep the time between the first and second peaks as short as possible.

Precision

Analyzing drugs with UV detection means that precision of retention times is of utmost importance. In addition, precision of peak areas must be less than 1 % in the low ng range to be compliant with official regulations. The precision of retention times and areas was determined using level 2 concentration. The results are shown in figure 3 and table 2. In figure 3 ten consecutive runs were overlaid.

Limit of detection

The limit of detection was calculated based on the chromatogram obtained for concentration level 7, see table 3. The limit of detection was in the low 3-digit pg range for ibuprofen and diclofenac, and in the low 2-digit pg range for paracetamol and benzoic acid.

Linearity

Linearity was tested using concentration levels 1 through 7. Figure 4 shows the linearity of paracetamol as an example. The results showed excellent linearity over the entire concentration range. Table 4 shows the correlation coefficients for all compounds.

Summary

The Agilent 1120 Compact LC was used for the analysis of pharmaceutical compounds. The instrument was able to analyze these compounds with high precision for retention times and areas. The precision for retention

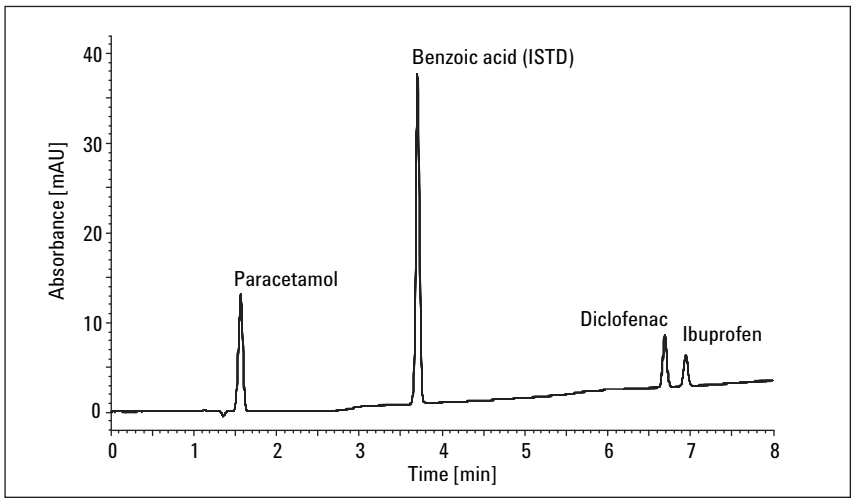


Figure 3
Precision of retention times and areas, showing overlay of 10 consecutive runs.

	% RSD Ret. Times	% RSD Areas
Paracetamol	0.060	0.297
Benzoic acid	0.022	0.179
Diclofenac	0.020	0.807
Ibuprofen	0.016	0.962

Table 2
Precision of retention times and areas for concentration level 2.

	Injected amount (3 µL injection)	Calculated LOD [ng]
Paracetamol	0.394	0.013
Benzoic acid	1.145	0.019
Diclofenac	0.394	0.197
Ibuprofen	0.596	0.298

Table 3
Limits of detection.

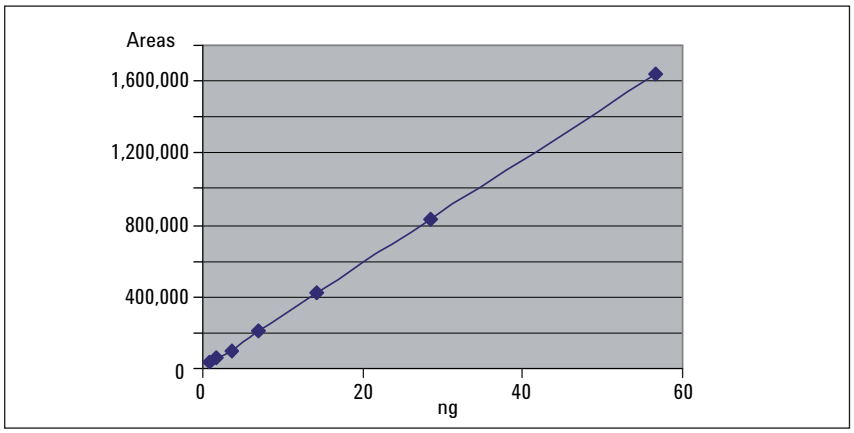


Figure 4
Linearity for paracetamol from 0.886 to 56.7ng per 3 µL injection.

	Correlation coefficient
Paracetamol	0.999966
Benzoic acid	0.999954
Diclofenac	0.999975
Ibuprofen	0.999910

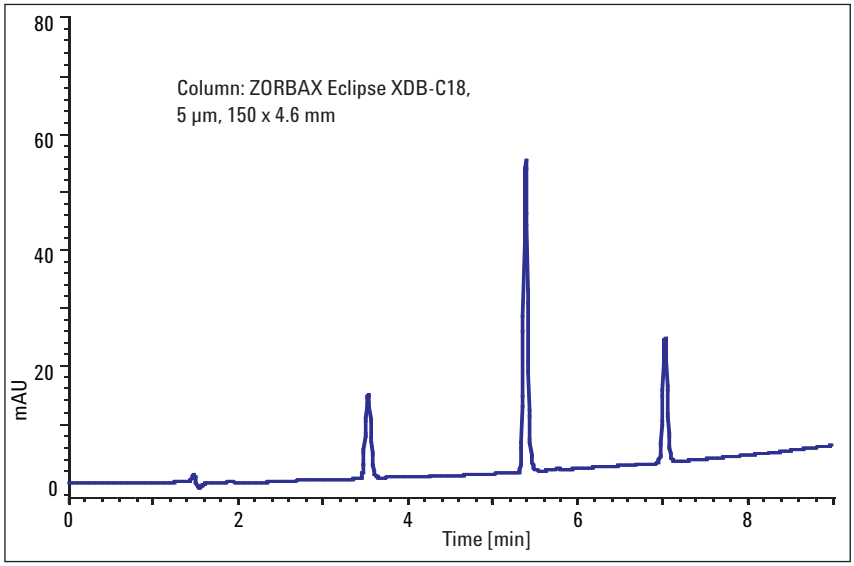
Table 4
Correlation coefficients.

times is less than 0.07 % RSD and less than 1.0 % RSD for areas of baseline separated peaks. The limits of detection were between 13 and 298 pg. The results showed excellent linearity over the tested concentration range and the correlation coefficient was between 0.999910 and 0.999975.

In-process control for Aripiprazole manufacturing with the Agilent 1120 Compact LC and ZORBAX C-18 columns

Application Note

Siji Joseph,
Patric Hörth



Abstract

The Agilent 1120 Compact LC is the system of choice for conventional, analytical-scale liquid chromatography. It is an integrated LC designed for ease of use, performance, and reliability. It is well-suited for in-process control during drug manufacturing, due to the highly precise retention times and peak areas.

This Application Note shows:

- Excellent retention time precision, with a relative standard deviation (RSD) < 0.07 %.
- Excellent area precision, with RSD < 0.25 % for baseline-separated peaks.

5989-8330EN

Introduction

During manufacturing of Active Pharmaceutical Ingredients (APIs) it is mandatory to monitor the levels of precursors or by-products that are formed during the chemical reactions. To ensure these criteria, manufacturing chemists have to carry out several analyses called in-process analyses. HPLC is commonly used as a technique, primarily because it separates individual analytes and also helps to perform qualitative and quantitative analysis. The developed chromatographic methods must be robust and reliable over a number of years.

The purpose of the present study was to develop an HPLC method for the analysis of Aripiprazole and its precursors with desired specificity and sensitivity. This Application Note describes the advantages obtained from in-process control of Aripiprazole by reversed-phase high-performance liquid chromatography (RP-HPLC). The power to carry out thorough in-process control of samples taken during the course of the entire process is highlighted.

Figure 1
Agilent 1120 Compact LC.

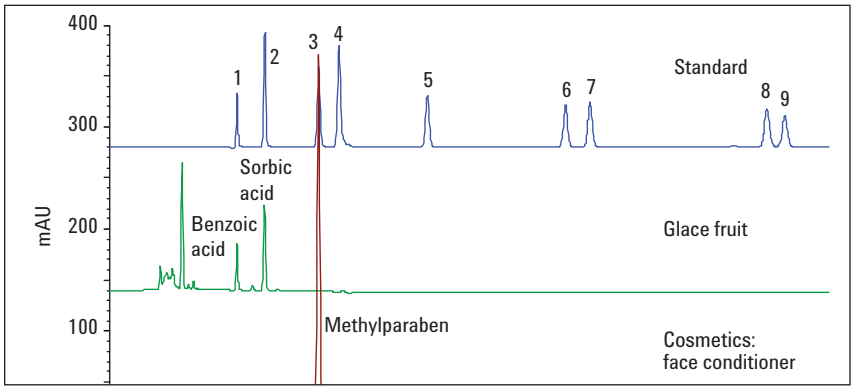


Figure 2
The synthesis of Aripiprazole from starting materials 1 and 2.

Experimental

Equipment

The Agilent 1120 Compact LC system included:

- A gradient pump with low-pressure mixing
- An autosampler with vial tray
- A column compartment for a column up to 250 mm in length
- A variable wavelength detector (VWD)

A ZORBAX Eclipse XDB C18, 5 µm, 150 x 4.6 mm, was used.

The instrument was controlled by Agilent EZChrome Elite Compact Compliance software.

Synthetic reaction

The antipsychotic drug Aripiprazole and its two starting materials were used for the study.
1-(2,3-Dichlorophenyl)piperazine hydrochloride (starting material-1) and 7-(4-bromobutoxy)-3,4-dihydrocarbostyril (starting material-2) are the two starting materials for the synthesis of Aripiprazole. The synthetic scheme is shown in figure 2.

Samples

In order to simulate in-process control samples, we prepared samples with varying concentrations of starting materials 1, 2 and the product Aripiprazole. During the reaction, the concentrations of the starting materials decrease, while the concentration of the product increases.

Chromatographic parameters

Experiments were performed to establish the best chromatographic conditions for the analysis of Aripiprazole and related compounds. Based on

these experiments, the following conditions were chosen:

- Sample: 1-(2,3-dichlorophenyl)pi-per-azine hydrochloride, 7-(4-bro-mobu-toxy)-3,4-dihydrocarbostyril and Aripiprazole
- Column: ZORBAX Eclipse XDB C18, 5 µm, 150 x 4.6 mm
- Mobile phase:
  A = water + 0.2 % trifluoroacetic acid (TFA),
  B = acetonitrile + 0.16 % TFA
  (The TFA improves retention and peak shape.)
- Flow rate: 1.0 mL/min
- Gradient: at 0 min 30 %B, at 7 min 70 %B, then hold the ratio for two more minutes
- Injection volume: 5 µL
- Autosampler programmed with a wash vial (acetonitrile) for rinsing exterior of the needle
- Run time: 9 min
- Post time: 5 min
- VWD: 254 nm,
  peak width (PW) > 0.05 min

Sequence table

Table 1 shows the sequence table that was created. The concentrations of the three compounds were varied from vial 2 to vial 9, and each sample was injected three times so that an RSD could be calculated.

Results and discussion

The chromatographic method was set up such that all compounds were baseline-separated. Figure 3 shows the separation of starting materials 1 and 2 and the product Aripiprazole. The separation time was nine minutes; the total run time (including equilibration) could be limited to 14 minutes, including re-equilibration time.

The chromatographic overlay of the reaction monitoring analysis is displayed in figure 4.

Line	Location	Sample name	# Injections	Injection volume (µL)
1	Vial 1	Blank	2	5
2	Vial 2	In-process control-1	3	5
3	Vial 3	In-process control-2	3	5
4	Vial 4	In-process control-3	3	5
5	Vial 5	In-process control-4	3	5
6	Vial 6	In-process control-5	3	5
7	Vial 7	In-process control-6	3	5
8	Vial 8	In-process control-7	3	5
9	Vial 9	In-process control-8	3	5

Table 1
Sequence table for the analysis.

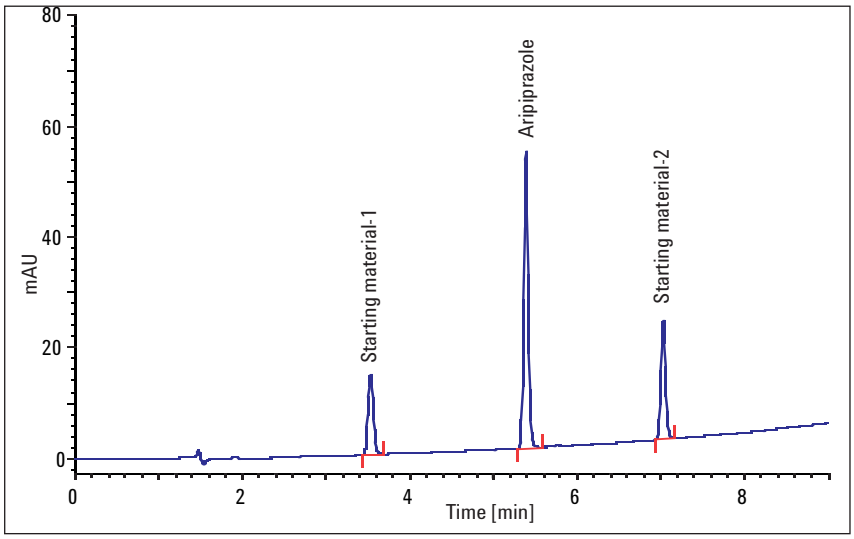


Figure 3
Chromatogram of Aripiprazole and starting materials 1 and 2, with retention times.

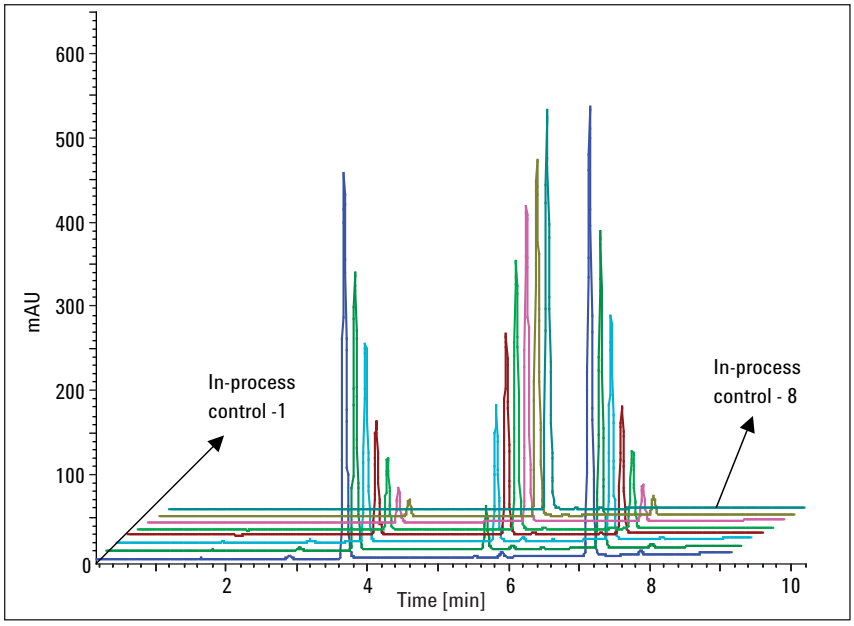


Figure 4
Chromatographic overlay of the in-process control samples, (x-axis offset).

Figure 5 represents the overlay of the three injections of the last in-process control sample of this series, plus a blank run. The in-process control sample contained Aripiprazole and starting material-1 at a level of 0.025 % of the main compound. The zoom-in of the starting material area is shown.

The assay precision (RSD) was assessed by expressing the standard deviation of repeated measurements as a percentage of the mean value. Intra-day precision was estimated from three replicates at eight levels of a standard mixture. The retention time and area precisions of three consecutive injections of each in-process check sample are summarized in table 2. The RSD for peak area was less than 0.25 %.

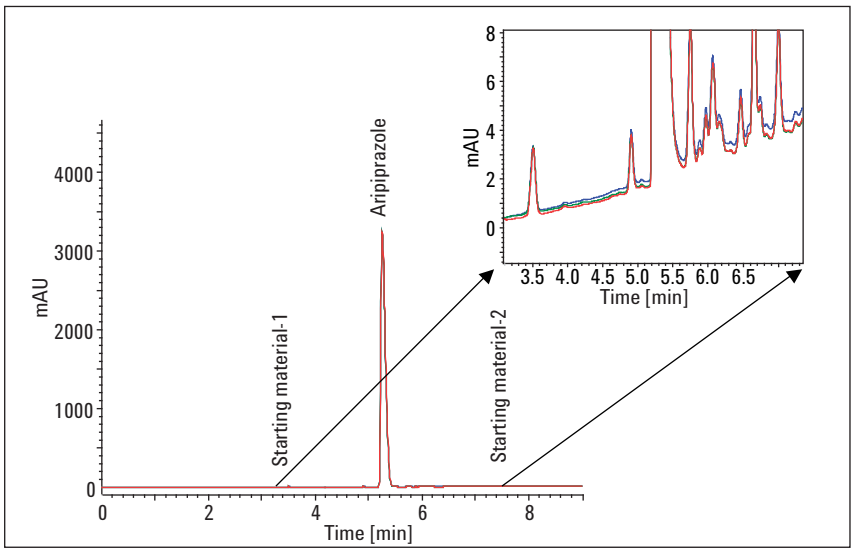


Figure 5
Zoomed chromatogram of 0.025 % starting material-1 with respect to main compound.

Sample	RSD of retention time			RSD of area		
	Starting material 1	Aripiprazole	Starting material 2	Starting material 1	Aripiprazole	Starting material 2
1	0.021 %	No peak	0.017 %	0.083 %	No peak	0.073 %
2	0.027 %	0.018 %	0.014 %	0.126 %	0.208 %	0.183 %
3	0.047 %	0.018 %	0.009 %	0.025 %	0.248 %	0.055 %
4	0.009 %	0.018 %	0.009 %	0.114 %	0.226 %	0.136 %
5	0.027 %	0.009 %	0.009 %	0.055 %	0.024 %	0.137 %
6	0.027 %	0.009 %	0.009 %	0.099 %	0.069 %	0.140 %
7	0.027 %	0.018 %	0.009 %	0.134 %	0.246 %	0.103 %
8	0.056 %	0.009 %	0.009 %	0.241 %	0.191 %	0.230 %

Table 2
Summary of the in-process analysis results.

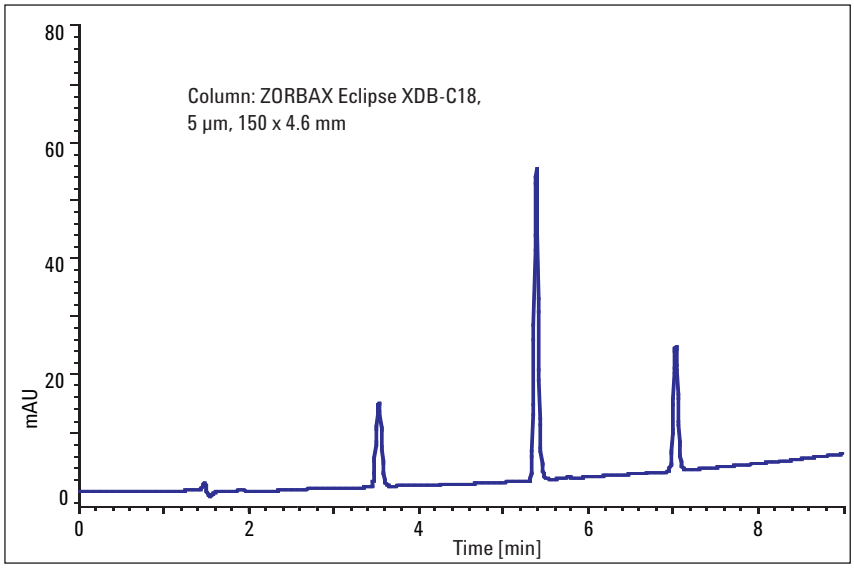
Conclusion

An HPLC method was developed for the simultaneous determination of Aripiprazole and potential precursor products. Furthermore, the chromatographic method was demonstrated to be useful for the in-process control of Aripiprazole synthesis. The Agilent 1120 Compact LC system is an excellent choice for in-process control, as it provides reproducible results with high precision for retention times and areas. For our example, the precision for retention times was < 0.06 % RSD and for areas of baseline-separated peaks was < 0.25 % RSD.

Detector linearity testing for Aripiprazole quality control with the Agilent 1120 Compact LC and ZORBAX C-18 columns

Application Note

Siji Joseph,
Patric Hörth



Abstract

The Agilent 1120 Compact LC is the system of choice for conventional, analytical-scale liquid chromatography. It is an integrated LC designed for ease of use, performance, and reliability. It is well-suited for the analysis of drugs due to highly precise retention times and peak areas and excellent detector linearity. This Application Note shows:

- Excellent retention time precision, with relative standard deviation (RSD) < 0.07 % for all six linearity levels.
- Excellent area precision, with RSD < 0.25 % for all six levels.
- Excellent linearity, with coefficient of correlation > 0.9999. This study covers a wide range of ~ 150 ng to 5,000 ng.

5989-8331EN

Introduction

In the development of an HPLC method for analysis, the detector plays a very important role as it defines the limits of concentrations over which the method can perform satisfactorily.

The purpose of this Application Note was to evaluate the detector linearity and precision of peak areas and retention times for the Agilent 1120 Compact LC system using the antipsychotic drug Aripiprazole (figure 2) as a test compound.

Figure 1
Agilent 1120 Compact LC.

Experimental

Equipment

The Agilent 1120 Compact LC system included:

- A gradient pump with low-pressure mixing
- An autosampler with vial tray
- A column compartment for a column up to 250 mm in length
- A variable wavelength detector (VWD)

A ZORBAX Eclipse XDB C18, 150 x 4.6 mm, 5 µm was used for all separations.

The instrument was controlled by Agilent EZChrome Elite Compact Compliance software.

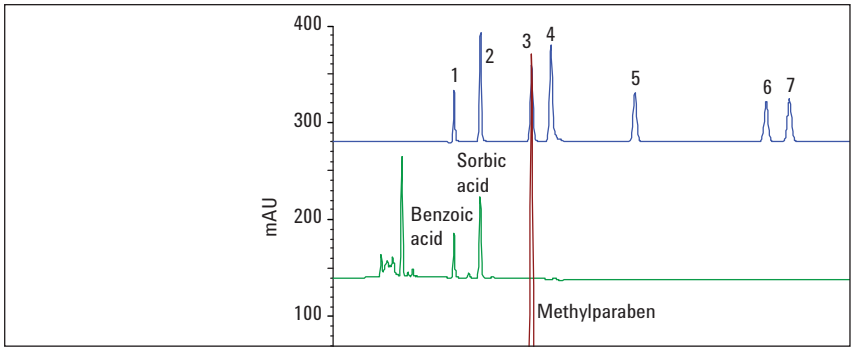


Figure 2
Structure of Aripiprazole.

Serial #	Weight or volume	Diluted to	Expected concentration	Solution name
1	20.00 mg of Aripiprazole	10 mL	2.000 mg/mL or 2,000 ng/µL	A (Stock)
2	500 µL of A	1 mL	1.000 mg/mL or 1,000 ng/µL	B, Level-6
3	500 µL of B	1 mL	0.500 mg/mL or 500 ng/µL	C, Level-5
4	250 µL of B	1 mL	0.250 mg/mL or 250 ng/µL	D, Level-4
5	125 µL of B	1 mL	0.125 mg/mL or 125 ng/µL	E, Level-3
6	62.5 µL of B	1 mL	0.0625 mg/mL or 62.5 ng/µL	F, Level-2
7	31.25 µL of B	1 mL	0.03125 mg/mL or 31.25 ng/µL	G, Level-1

Note: To prepare the stock solution, Aripiprazole was initially dissolved in methanol (20 % of the total make-up volume) and the solution was then made up to the mark with diluent.

Table 1
Sample preparation.

Chromatographic parameters

The chromatographic conditions were as follows:

- Sample: Aripiprazole
- Column: ZORBAX Eclipse XDB C18, 5 µm, 150 x 4.6 mm,
- Mobile phase:
  A = water + 0.2 % TFA,
  B = acetonitrile + 0.16 % TFA
- Flow rate: 1.0 mL/min
- Gradient: at 0 min 30 %B, at 7 min 70 %B, then hold the ratio for another two minutes
- Injection volume: 5 µL
- Autosampler programmed with a wash vial (using acetonitrile) for rinsing exterior of the needle
- Run time: 9 min
- Post time: 5 min
- Column oven: 40 °C

- VWD: 254 nm, peak width (PW) > 0.05 min
- Diluent / blank: 60:40 acetonitrile:water

Sample preparation
The samples for the linearity test were prepared as per table 1.

Sequence table
Table 2 shows the sequence table that was set up in the Agilent EZChrom Elite Compact Compliance software.

Results and discussion

Figure 3 shows the chromatogram of Aripiprazole. The mobile phase contained trifluoroacetic acid as modifier, which improved retention and peak shape.

Figure 4 shows the chromatographic overlay of all six linearity levels. The results of the linearity results are summarized in table 3, and the linearity plot is displayed in figure 5. The observed linearity correlation was r-squared > 0.9999.

Line	Location	Sample name	# Injections	Injection volume (µL)
1	Vial 1	Blank	2	5
2	Vial 2	Linearity Level-1	6	5
3	Vial 3	Blank	2	5
4	Vial 4	Linearity Level-2	6	5
5	Vial 5	Blank	2	5
6	Vial 6	Linearity Level-3	6	5
7	Vial 7	Blank	2	5
8	Vial 8	Linearity Level-4	6	5
9	Vial 9	Blank	2	5
10	Vial 10	Linearity Level-5	6	5
11	Vial 11	Blank	2	5
12	Vial 12	Linearity Level-6	6	5

Table 2
Sequence table.

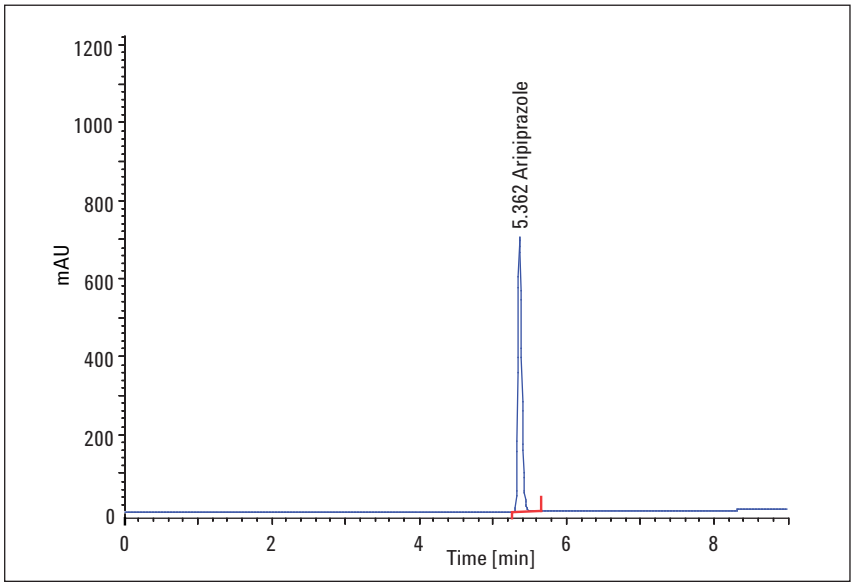


Figure 3
Chromatogram of Aripiprazole.

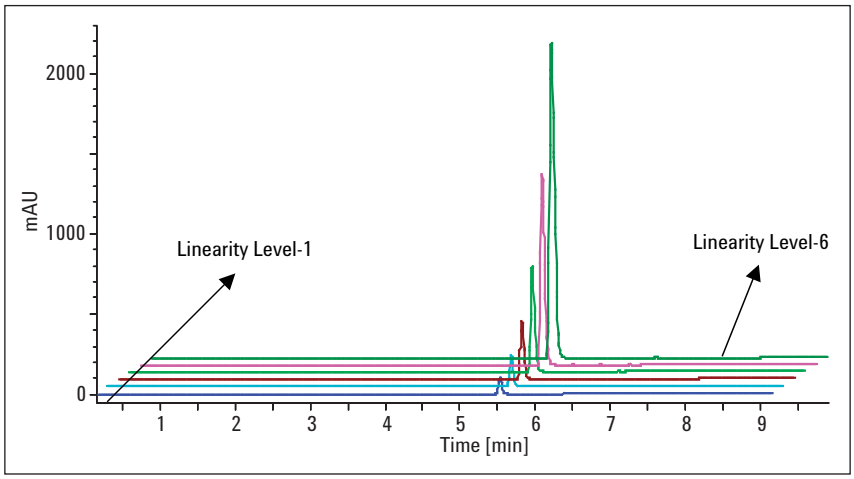


Figure 4
Overlay of the chromatograms of all six concentration levels (with time and absorbance offset).

Conclusion

The variable wavelength detector within the Agilent 1120 Compact LC gives excellent linearity over a very wide range of concentrations. The instrument is able to analyze Aripiprazole with high precision for retention times and peak areas. In this study, the precision for retention times was < 0.03 % RSD and for areas of baseline-separated peaks was < 0.24 % RSD.

The Agilent 1120 Compact LC system is well-suited for this application, as it delivers the needed data quality and is based on a proven robust design. This Application Note also demonstrates that the Agilent 1120 Compact LC system meets the detector linearity requirements of a pharmaceutical QA/QC lab.

Results	Level-1	Level-2	Level-3	Level-4	Level-5	Level-6
RSD of area	0.243%	0.159%	0.126%	0.218%	0.124%	0.210%
RSD of retention time	0.017%	0.023%	0.025%	0.020%	0.022%	0.020%

Table 3
Linearity results.

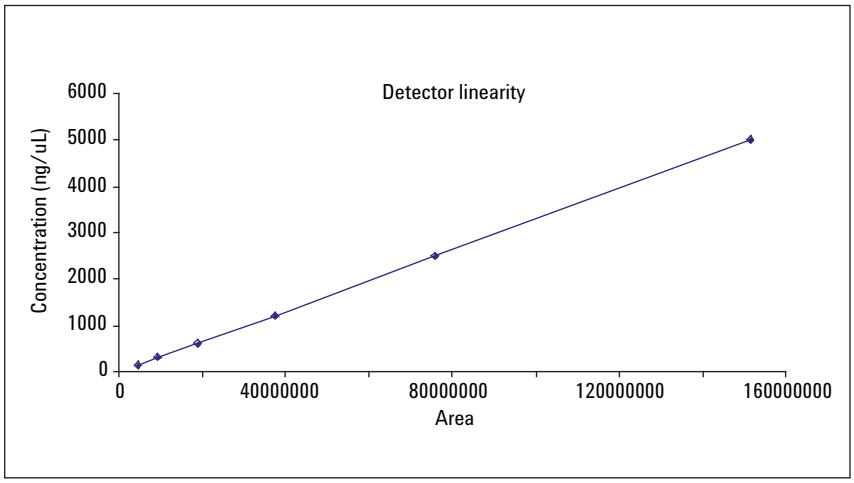
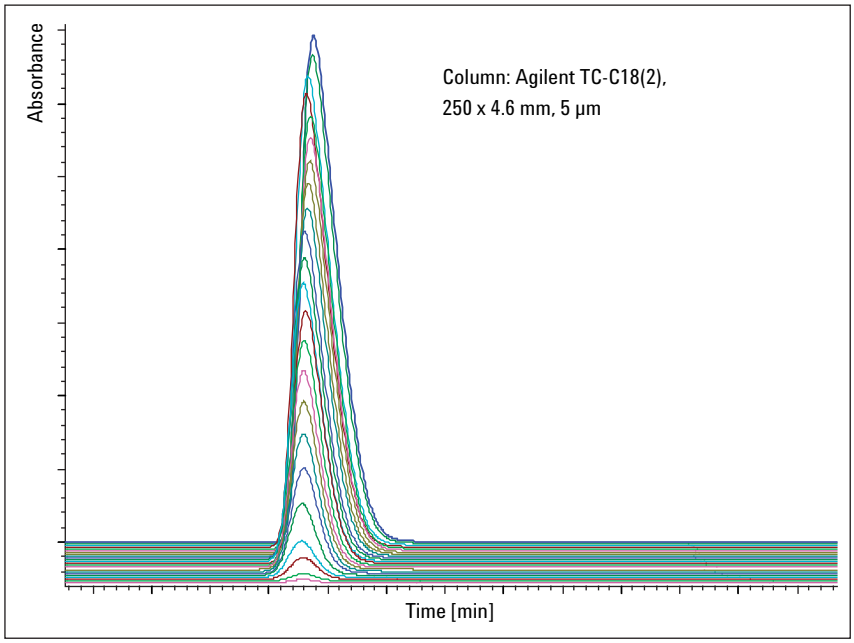


Figure 5
Linearity curve for Aripiprazole from~ 150 to 5,000 ng injected.

Injector linearity testing for Tramadol quality control with the Agilent 1120 Compact LC and Agilent TC C18(2) columns

Application Note

Siji Joseph,
Patric Hörth



Abstract

The Agilent 1120 Compact LC is the system of choice for conventional, analytical-scale liquid chromatography. It is an integrated LC designed for ease of use, performance, and reliability. It is well-suited for the analysis of drugs due to the highly precise retention times and peak areas, excellent detector linearity, and excellent injector linearity. This Application Note shows:

- Excellent retention time precision, with relative standard deviation (RSD) < 0.07 % for all 22 linearity levels.
- Excellent area precision, with RSD < 0.5 % for all 22 levels.
- Excellent injector linearity, with coefficient of correlation (R^2) > 0.9999.

This study covers a wide range of 1 µL to 100 µL injected, and the solution concentration used was 100 ng/µL Tramadol.

5989-8335ENA

Introduction

For the analysis of drugs in routine QA/QC, it is very important to have highly precise, accurate, linear, and robust LC systems. The performance of the autosampler plays a major role in the accuracy of the analysis. The design of the Agilent 1120 Compact LC autosampler is well known for its accuracy and precision in injection. It delivers the needed data quality and is based on a proven robust design. In this Application Note, the linearity performance of the Agilent 1120 Compact LC autosampler is demonstrated via injections of Tramadol, an analgesic.

Figure 1
Agilent 1120 Compact LC.

Experimental

Equipment

The Agilent 1120 Compact LC system included:

- A gradient pump with low-pressure mixing
- An autosampler with vial tray (maximum injection volume is 100 µL)
- A column compartment for a column of up to 250 mm in length
- An Agilent TC (Typical Carbon load) C18(2) column, 250 x 4.6 mm, 5 µm
- A variable wavelength detector (VWD)

The instrument was controlled by Agilent EZChrom Elite Compact software.

Sample preparation

Stock solution: A stock solution of 1 mg/mL Tramadol was prepared.

Linearity test sample (diluted stock solution): 1 mL of the stock solution was diluted to 10 mL, resulting in a concentration of 0.1 mg/mL (100 ng/µL).

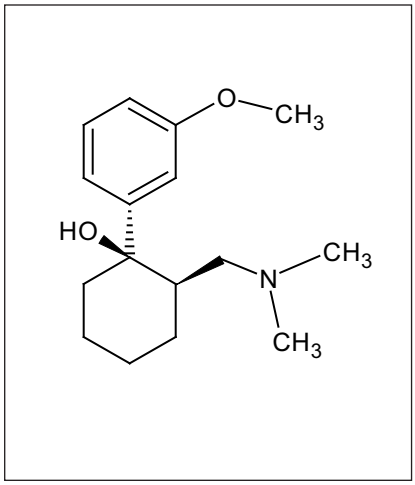


Figure 2
Chemical structure of Tramadol.

Chromatographic parameters

A chromatographic method was developed to separate Tramadol and its potential impurities.
This method was then used (with Tramadol alone) for the injector linearity tests. The conditions were:

- Sample: Tramadol
- Column: Agilent TC-C18(2), 250 x 4.6 mm, 5 µm
- Mobile phases: A = water + 0.2 % TFA, B = acetonitrile + 0.16 % TFA
- Flow rate: 1.2 mL/min
- Gradient: at 0 min 30 %B, at 9 min 85 %B, then hold the ratio for three more minutes
- Injection volume: varied from 1 µL to 100 µL
- Autosampler programmed with a wash vial
- Run time: 12 min
- Post time: 5 min
- Column oven: 30 °C
- VWD: 270 nm, peak width (PW) > 0.05 min
- Diluent / blank: 30:70 acetonitrile:water

Sequence table

The linearity test sample was injected as per the sequence table shown as table 1.

Results and discussion

Figure 3 shows an example chromatogram of Tramadol from the injector linearity test. The mobile phase contained trifluoroacetic acid as modifier, which improved retention and peak shape.

Serial #	Sample name	Injection volume (µL)	# Injections
1	Blank	5	2
2	Linearity Level:1	1	3
3	Linearity Level:2	3	3
4	Linearity Level:3	5	3
5	Linearity Level:4	10	3
6	Linearity Level:5	15	3
7	Linearity Level:6	20	3
8	Linearity Level:7	25	3
9	Linearity Level:8	30	3
10	Linearity Level:9	35	3
11	Linearity Level:10	40	3
12	Linearity Level:11	45	3
13	Linearity Level:12	50	3
14	Linearity Level:13	55	3
15	Linearity Level:14	60	3
16	Linearity Level:15	65	3
17	Linearity Level:16	70	3
18	Linearity Level:17	75	3
19	Linearity Level:18	80	3
20	Linearity Level:19	85	3
21	Linearity Level:20	90	3
22	Linearity Level:21	95	3
23	Linearity Level:22	100	3

Table 1
The sequence table.

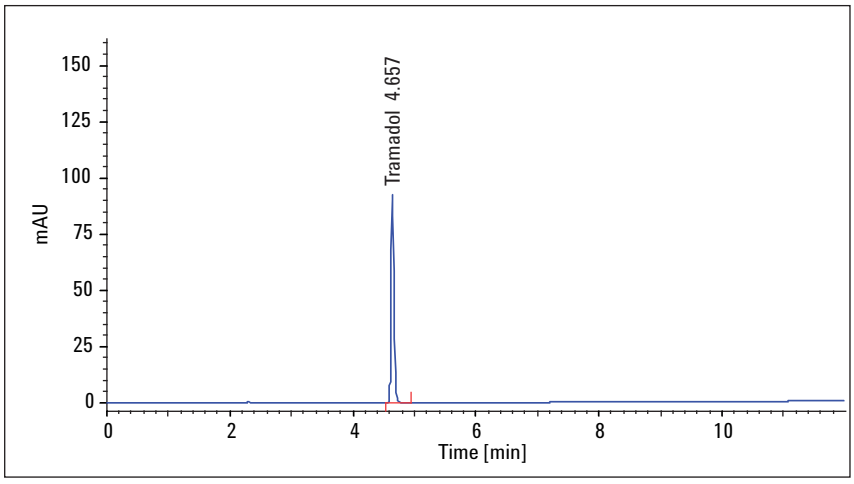


Figure 3
Chromatogram of Tramadol.

Table 2 lists the average peak heights and peak areas for each concentration that was injected.

The chromatographic overlay of all linearity levels is shown in figure 4.

Sample name	On-column concentration (µg)	Peak height	Peak area
Linearity Level:1	0.1	157336	522936
Linearity Level:2	0.3	472170	1570002
Linearity Level:3	0.5	786250	2622929
Linearity Level:4	1.0	1553884	5244121
Linearity Level:5	1.5	2289525	7857707
Linearity Level:6	2.0	3000436	10471135
Linearity Level:7	2.5	3680418	13077010
Linearity Level:8	3.0	4338134	15685629
Linearity Level:9	3.5	4974238	18305228
Linearity Level:10	4.0	5584654	20913229
Linearity Level:11	4.5	6186702	23513327
Linearity Level:12	5.0	6750352	26119986
Linearity Level:13	5.5	7323479	28725172
Linearity Level:14	6.0	7800501	31103171
Linearity Level:15	6.5	8328025	33699927
Linearity Level:16	7.0	8833069	36264800
Linearity Level:17	7.5	9328439	38847902
Linearity Level:18	8.0	9811331	41437670
Linearity Level:19	8.5	10317715	44113978
Linearity Level:20	9.0	10734428	46673257
Linearity Level:21	9.5	11175523	49272540
Linearity Level:22	10	11601457	51871167

Table 2
Tabulation of peak areas and heights.

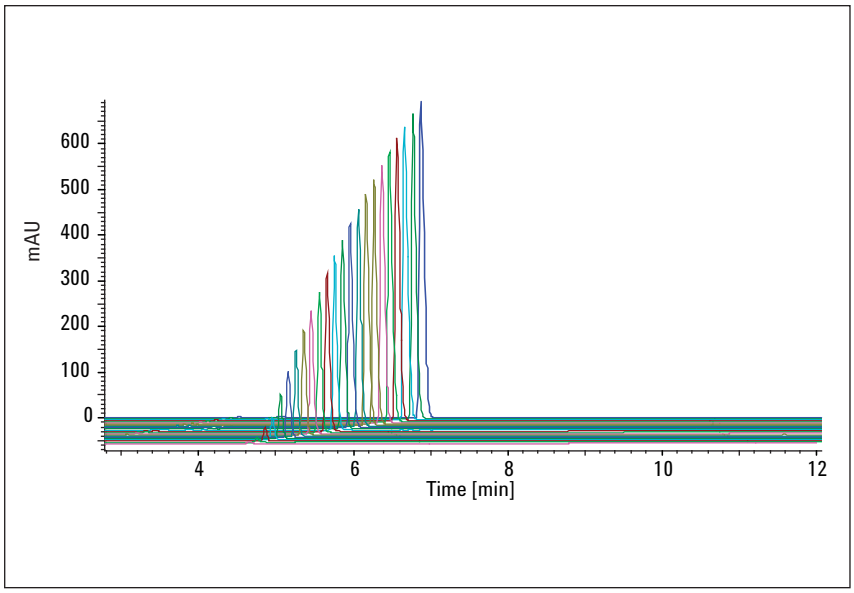


Figure 4
Chromatographic overlay of all linearity levels.

The linearity plot is displayed in figure 5.

Figure 6 shows the % RSD of retention times for each linearity level.

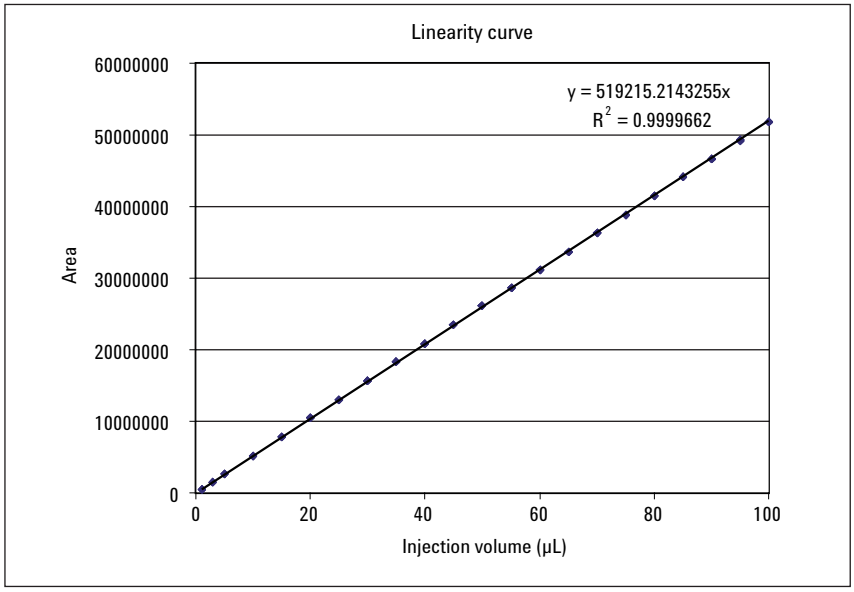


Figure 5
Injector linearity of the Agilent 1120 Compact LC using 1 µL to 100 µL of a solution of Tramadol. The observed linearity correlation (R2) is > 0.9999.

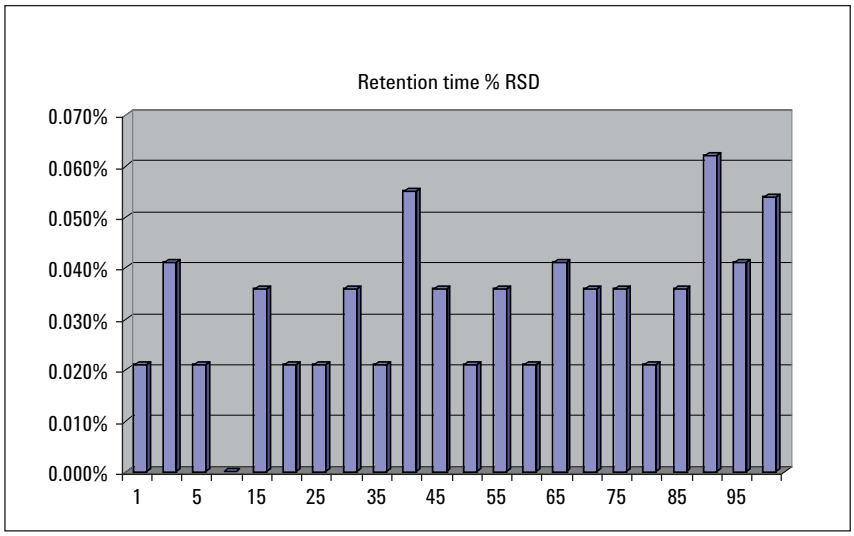


Figure 6
% RSDs for retention times. RSDs of all levels are within the limit of < 0.07 %.

Figure 7 shows the % RSD of the peak areas for each linearity level.

Conclusion

The autosampler that is integrated into the Agilent 1120 Compact LC gives excellent injection precision over a wide range of injection volumes (1 µL to 100 µL), and is a best choice for pharmaceutical QA/QC and drug discovery. Our study with Tramadol shows that the system is able to analyze this compound with high precision for retention times (< 0.07 % RSD) and for peak areas (< 0.5 % RSD).

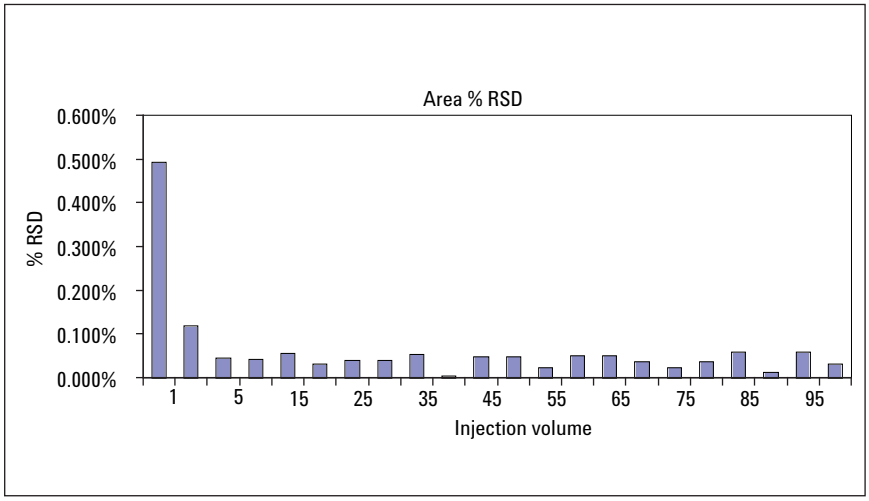
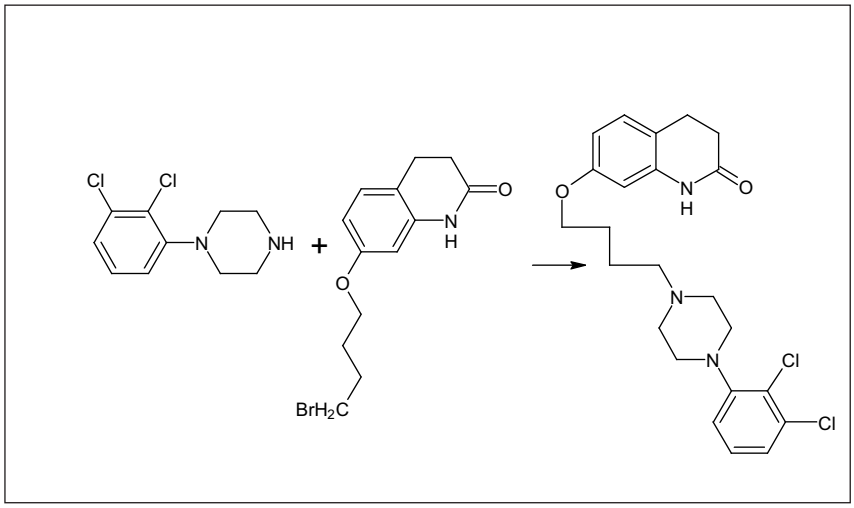


Figure 7
% RSDs of peak areas. RSDs of peak areas for all the levels are within the limit of < 0.5 %.

System suitability testing for Aripiprazole quality control with the Agilent 1120 Compact LC and ZORBAX C-18 columns

Application Note

Siji Joseph,
Patric Hörth



Abstract

The Agilent 1120 Compact LC is the system of choice for conventional, analytical-scale liquid chromatography. It is an integrated LC designed for ease of use, performance, and reliability. It is well-suited for the analysis of drugs due to highly precise retention times and peak areas and low detection limits. This Application Note shows:

- Excellent retention time precision, with relative standard deviation (RSD) < 0.07 %.
- Excellent area precision, with RSD < 0.25 % for baseline-separated peaks.
- Excellent height precision, with RSD < 0.25 % for baseline-separated peaks.

5989-8333EN

Introduction

In the pharmaceutical industry, liquid chromatography is a versatile tool for separation of individual analytes. Innumerable methods have been developed for analysis on HPLC as it is proven to be very reliable and reproducible at all stages from drug discovery to manufacturing. As a result, it is necessary to verify that any HPLC system performs within an acceptable range of precision and accuracy every day.

System Suitability Testing (SST) is a measure of instrument performance on a day-to-day basis. These tests ensure that the method and the HPLC system can generate results of acceptable accuracy and precision. The criteria selected is based on critical chromatographic parameters such as resolution, reproducibility in retention time, peak area and height, column efficiency and their variation (Standard Deviation) within acceptable limits which are defined during the method validation experiments.

In this Application Note, we focus on this final validation step and evaluate the suitability of the Agilent 1120 Compact LC system for the analysis of Aripiprazole and its precursors.

Figure 1
Agilent 1120 Compact LC.

Experimental

Equipment

The Agilent 1120 Compact LC system included:

- A gradient pump with low-pressure mixing
- An autosampler with vial tray
- A column compartment for a column up to 250 mm in length
- A variable wavelength detector (VWD)

An Agilent ZORBAX Eclipse XDB C18, 5µm, 150 x 4.6 mm was used

The instrument was controlled by Agilent EZChrome Elite Compact Compliance software.

Compounds

1-(2,3-Dichlorophenyl)piperazine hydrochloride (starting material-1) and 7-(4-bromobutoxy)-3,4-dihydrocarbostyril (starting material-2) are the two starting materials for the synthesis of Aripiprazole. The synthetic scheme is shown in figure 2. For this study, the two starting materials are treated as impurities.

Preparation of samples

Stock preparation: 2 mg/mL of the Aripiprazole and 5 mg/mL of starting material-1 and -2 were prepared as individual stock solutions. Aripiprazole and the two starting materials were initially dissolved in methanol (20 % of the total make-up volume), and diluent was then added up to the marks.

System suitability sample: A test mix for system suitability was prepared with 10 µg/mL Aripiprazole and each of the impurities at 5 µg/mL.

Chromatographic parameters

The chromatographic method was set up such that all compounds were baseline-separated. The conditions were:

- Sample: 1-(2,3-dichlorophenyl)pi-perazine, 7-(4-bromobutoxy)-3,4-dihydrocarbostyril, and Aripiprazole
- Column: Agilent ZORBAX Eclipse XDB C18, 5µm, 150 x 4.6 mm
- Mobile phases: A = water + 0.2 % trifluoroacetic acid (TFA), B = acetonitrile + 0.16 % TFA
- Flow rate: 1.0 mL/min
- Gradient: at 0 min 30 %B, at 7 min 70 %B, then hold the ratio for two more minutes
- Injection volume: 10 µL
- Autosampler programmed with a wash vial (using acetonitrile) for rinsing exterior of the needle
- Run time: 9 min
- Post time: 5 min
- Column oven: 40 °C
- VWD: 254 nm, peak width (PW) > 0.05 min
- Diluent / blank: 60:40 acetonitrile:water

Sequence table
Based on the recommendations by ICH (International Conference on Harmonization) for system suitability tests, the sequence table shown as table 1 was set up in the Agilent EZChrom Elite Compact Compliance software.

Results and discussion

In figure 3, an example chromatogram for system suitability testing shows excellent resolution. The separation time was nine minutes; the total run time (including the re-equilibration) could be limited to 14 minutes. The mobile phase contained trifluoroacetic acid as modifier, which improved retention and peak shape.

When analyzing drugs with UV detection, precision of retention times is of utmost importance. The precision of retention times and areas was determined from 10 replicate injections of system suitability sample. Figure 4 shows an overlay of 10 consecutive runs.

The acceptance criteria for this system suitability study are tabulated in table 2.

Parameter	Limit
RSD of retention time (RT)	< 0.07 %
RSD of area	< 1.00 %
Resolution	> 2.00
Asymmetry	< 2.00
Theoretical plates	> 2000
Peak width	< 0.08 min
RSD of height	< 0.50 %

Table 2
Acceptance criteria.

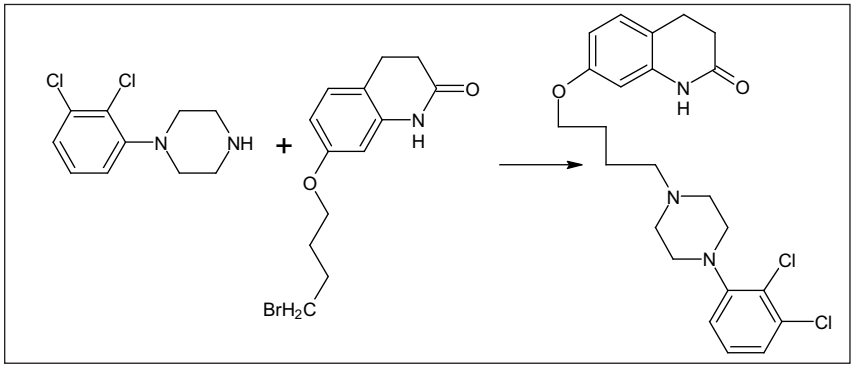


Figure 2
Structures of starting material-1 and -2 and the product Aripiprazole.

Line	Location	Sample name	# Injections	Injection volume (µL)
1	Vial 1	Blank	3	10
2	Vial 2	System suitability	10	10
3	Vial 3	Blank	1	10

Table 1
Sequence table.

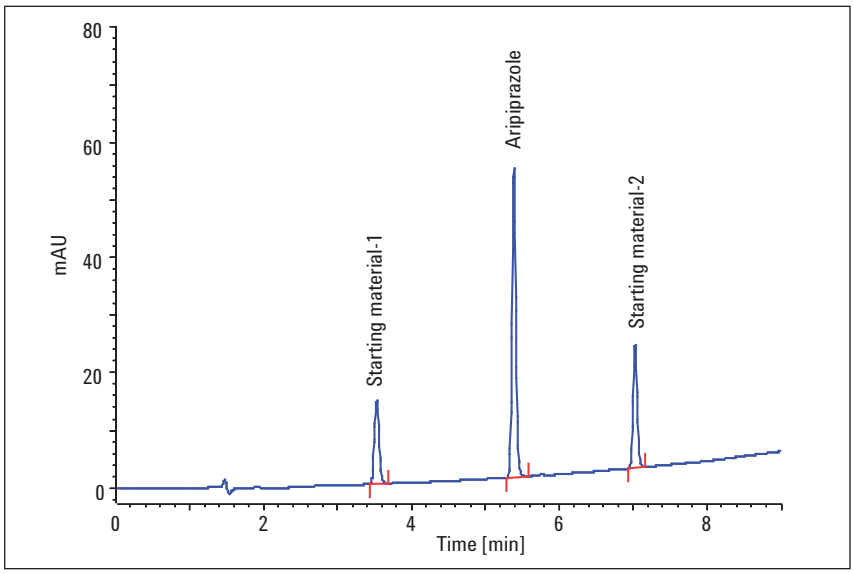


Figure 3
Chromatogram of Aripiprazole and its impurities.

Compound	Amount (µg/mL)	RSD of RT (%)	RSD of area (%)	Reso-lution	PW	Asymmetry	Theoretical plates	RSD of height (%)	Passed (yes/no)
Starting material-1	5.15	0.033	0.189	NA	0.07	1.02470	>14000	0.228	Yes
Aripiprazole	10.1	0.027	0.129	17.87	0.05	1.14031	>58000	0.183	Yes
Starting material-2	5.4	0.021	0.137	17.48	0.06	1.09432	>60000	0.169	Yes

*N/A = not applicable

Table 3
System suitability test results.

The results of the system suitability testing are shown in figure 4 and are summarized in table 3.

These results of the system suitability test for Aripiprazole demonstrate that the Agilent 1120 Compact LC meets the stringent performance requirements for pharmaceutical QA/QC analysis.

Conclusion

For the analysis of drugs in routine QA/QC, it is very important to have highly precise, accurate, and robust LC systems. This enables reliable analysis of pharmaceutical drugs and their impurities. The Agilent 1120 Compact LC system is well-suited for this application because it delivers the needed data quality and is based on a proven robust design.

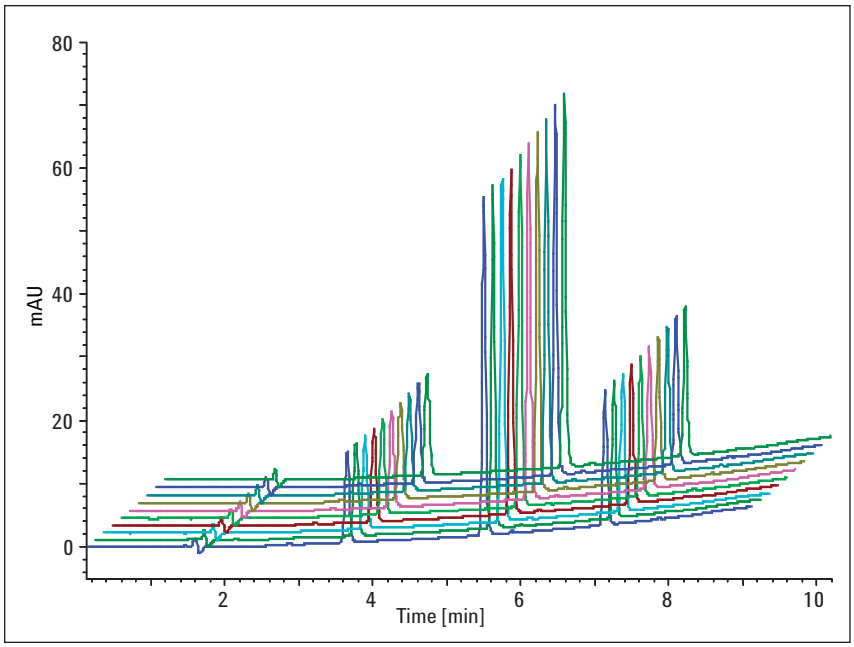
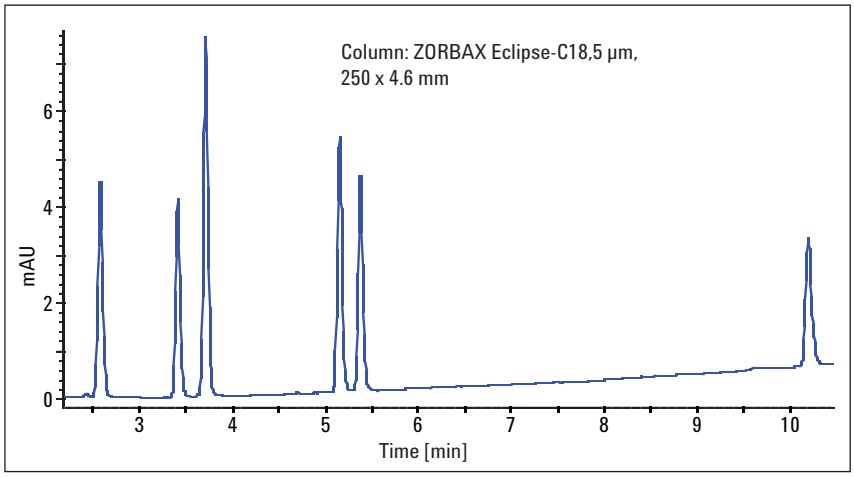


Figure 4
Overlay of 10 repetitive chromatograms.

System suitability testing for Tramadol quality control with the Agilent 1120 Compact LC and ZORBAX C-18 columns

Application Note

Siji Joseph,
Patric Hörth



Abstract

The Agilent 1120 Compact LC is the system of choice for conventional, analytical-scale liquid chromatography. It is an integrated LC designed for ease of use, performance, and reliability. It is well-suited for the analysis of drugs due to the highly precise retention times and peak areas. This Application Note shows:

- Excellent retention time precision, with relative standard deviation (RSD) < 0.07 %.
- Excellent area precision, with RSD < 0.25 % for baseline-separated peaks.
- Excellent height precision, with RSD < 0.25 % for baseline-separated peaks.

5989-8334EN

Introduction

System Suitability Testing (SST) is a measure of instrument performance on a day-to-day basis. These tests ensure that the method and the HPLC system can generate results of acceptable accuracy and precision. The criteria selected is based on critical chromatographic parameters such as resolution, reproducibility in retention time, peak area and height, column efficiency and their variation (Standard Deviation) within acceptable limits which are defined during the method validation experiments. Currently SST measurements have become a part of the analytical procedures, and are also recommended by the pharmacopeias and accepted by the United States Food and Drug Administration (FDA).

In this Application Note, we focus on this final validation step and evaluate the suitability of the Agilent 1120 Compact LC system for the analysis of the analgesic drug Tramadol and potential impurities from its production.

Figure 1
Agilent 1120 Compact LC.

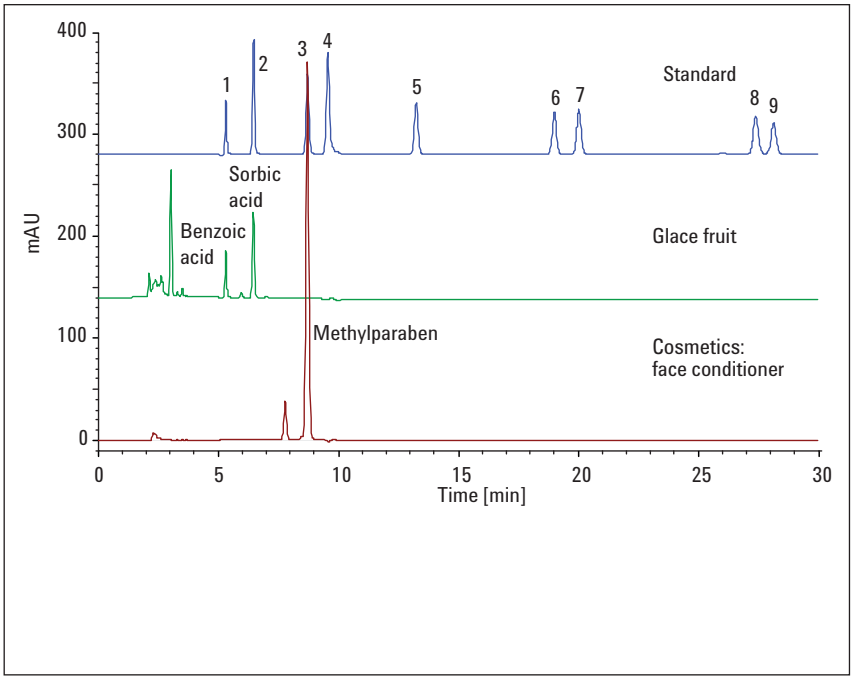


Figure 2
Structures of the compounds from the synthesis of Tramadol.

Experimental

Equipment

The Agilent 1120 Compact LC system included:

- A gradient pump with low-pressure mixing
- An autosampler with vial tray
- A column compartment for a column up to 250 mm in length
- A variable wavelength detector (VWD)

A ZORBAX Eclipse XDB C18, 5 µm, 250 x 4.6 mm, was used.

The instrument was controlled by Agilent EZChrome Elite Compact Compliance software.

Structures of compounds used

Figure 2 shows the synthesis of Tramadol, highlighting both starting materials and potential byproducts.

This study focused on analysis of all of these compounds except for impurity E, which is an ultraviolet (UV)-inactive starting material.

Sample preparation

Stock preparation: 2 mg/mL of Tramadol and 5 mg/mL each of starting material (3-bromoanisole) and impurities A, B, C, and D were prepared as the six stock solutions.

System suitability sample: A test mix for system suitability was pre-pared with Tramadol at 10 µg/mL and all other starting materials/impurities at 5 µg/mL each. This test mix was injected six times for the calculation of system suitability.

Chromatographic parameters

The chromatographic method was set up such that all compounds were baseline-separated. The conditions were:

- Sample: Tramadol; impurities A, B, C, and D; and 3-bromoanisole
- Column: ZORBAX Eclipse XDB C18, 5 µm, 250 x 4.6 mm,
- Mobile phases:
  A = water + 0.2 % TFA,
  B = acetonitrile + 0.16 % TFA
- Flow rate: 1.2 mL/min
- Gradient: at 0 min 30 %B, at 9 min 85 %B, then hold the ratio for three more minutes
- Injection volume: 10 µL
- Autosampler programmed with a wash vial (using acetonitrile) for cleaning the needle exterior
- Run time: 12 min
- Post time: 5 min
- Column oven: 30 °C
- VWD: 270 nm,
  peak width (PW) > 0.05 min
- Diluent / blank: 30:70
  acetonitrile:water

Sequence table

Based on the recommendations by ICH (International Conference on Harmonization) for system suitability performance tests, the sequence table shown as table 1 was set up in the Agilent EZChrom Elite Compact software.

Line	Location	Sample name	# Injections	Injection volume (µL)
1	Vial 1	Blank	3	10
2	Vial 2	System suitability	6	10
3	Vial 3	Blank	1	10

Table 1
Sequence table.

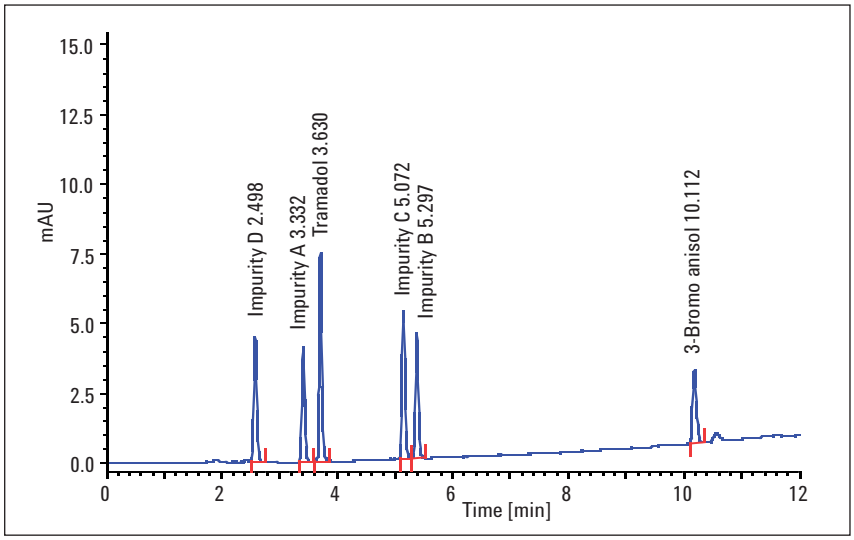


Figure 3
Chromatogram of Tramadol with impurities and starting material.

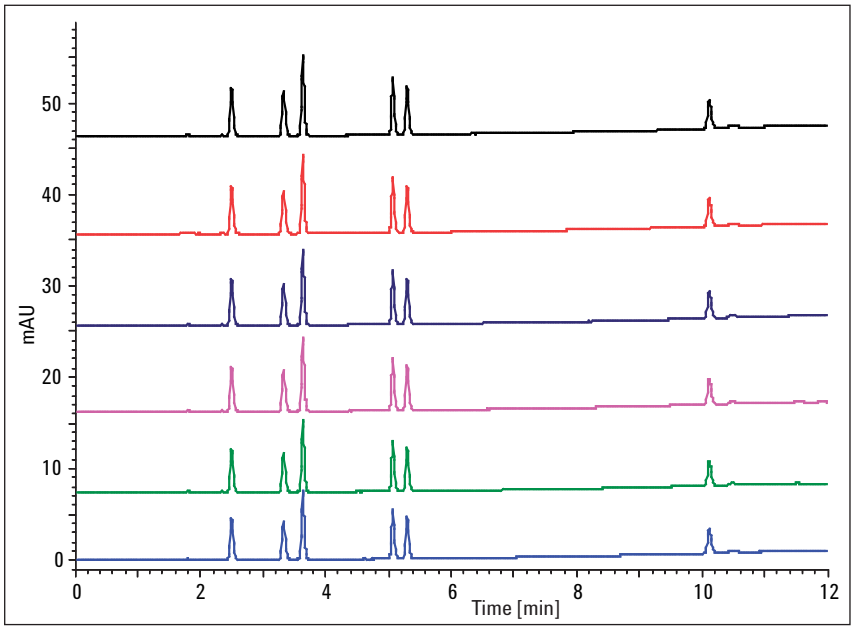


Figure 4
Overlay of 6 repetitive chromatograms..

Results and discussion

In figure 3, an example chromatogram for system suitability testing shows excellent resolution. The separation time was 12 minutes and the total run time (including time for re-equilibration) could be limited to 17 minutes. The mobile phase contained trifluoroacetic acid as modifier, which improved peak shape.

When analyzing drugs with UV detection, precision of retention times is of utmost im-portance. The precision of retention times and areas was determined from the six replicate injections of system suitability sample. Figure 4 shows an overlay of six consecutive runs.

The acceptance criteria for this system suitability study are tabulated in table 2.

Parameter	Limit
RSD of retention time (RT)	< 0.07 %
RSD of area	< 1.00 %
Resolution	> 2.00
Asymmetry	< 2.00
Theoretical plates	> 2000
Peak width	< 0.08 min
RSD of height	< 0.50 %

Table 2
Acceptance criteria.

The results of the system suitability testing are shown in figure 4 and are summarized in table 3. These results of the system suitability test for Tramadol demonstrate that the Agilent 1120 Compact LC meets the stringent performance requirements for pharmaceutical QA/QC analysis.

Compound	Results on 250 x 4.6 mm Agilent TC-C18 5 µm column								Passed
	Amount (µg/mL)	RSD of RT (%)	RSD of area (%)	Resolution	PW (min)	Asymmetry	Theoretical plates	RSD of height (%)	(yes/no)
Tramadol	10.1	0.037	0.150	3.23	0.05	1.16130	>25000	0.207	Yes
Impurity A	5.2	0.068	0.234	8.91	0.05	1.14739	>20000	0.347	Yes
Impurity B	5.3	0.013	0.191	2.47	0.05	1.14858	>50000	0.439	Yes
Impurity C	5.2	0.017	0.165	15.83	0.05	1.17513	>50000	0.199	Yes
Impurity D	5.1	0.066	0.237	NA	0.05	1.16080	>11500	0.413	Yes
3-Bromoanisole	5.2	0.008	0.212	45.17	0.07	1.28923	>60000	0.281	Yes

*N/A = not applicable

Table 3
System suitability test results.

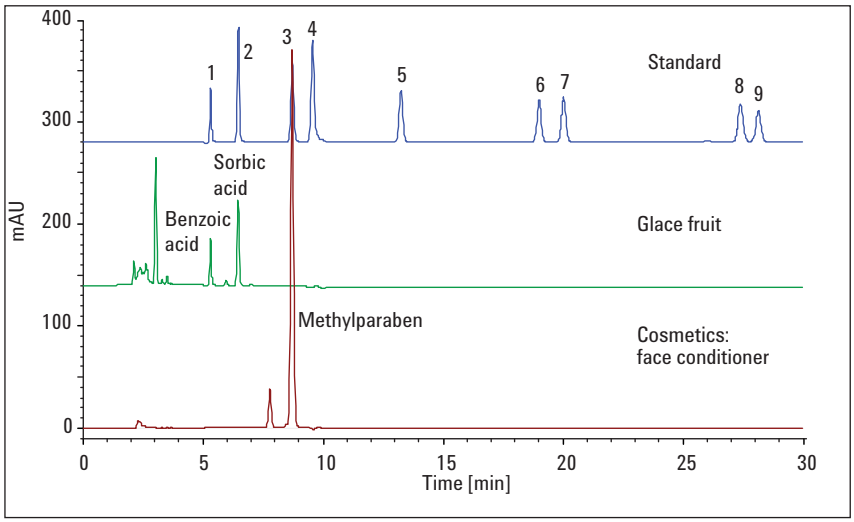
Conclusion

The Agilent 1120 Compact LC is ideally suited for QA/QC of pharmaceuticals because the system gives excellent precision for retention times and areas. In this study, the precision for retention times was < 0.07 % RSD and for areas of baseline-separated peaks was < 0.25 % RSD.

Development and validation of an HPLC method to analyze ibuprofen and impurities according to the European Pharmacopoeia

Application Note

Detlef Wilhelm
ANATOX GmbH & Co. KG, Germany



Abstract

This Application Note describes the development of a fast, accurate, and reproducible method to analyze ibuprofen and related impurities according to European Pharmacopoeia (EP) regulations[1], using an Agilent 1120 Compact LC. The experiments described in this Application Note include determination of precision of areas and retention times, as well as chromatographic parameters like resolution and signal-to-noise ratios. The experiments prove precise results from a system that was optimized for everyday productivity, and they fulfill regulatory compliance.

5989-9241EN

Introduction

Performing routine testing in a quality control laboratory with standardized methods for active pharmaceutical ingredients (APIs) or final products requires analytical instrumentation with high reliability and ease-of-use, combined with optimal cost-of-ownership.

This Application Note shows how the Agilent 1120 Compact LC[2], a highly robust and reliable instrument for standard LC methodology, can be used effectively in a routine environment to efficiently measure pharmaceutical compounds such as ibuprofen and related impurities.

Ibuprofen impurities A, B, C, D, and E were analyzed on an Agilent 1120 Compact LC system according to methodology described in the EP, and system suitability and performance tests were executed.

Experimental

Equipment

An Agilent 1120 Compact LC system with the following built-in modules was used:

- Gradient pump and vacuum degasser
- Autosampler
- Column oven
- Variable wavelength detector

Preparation of samples

The impurities A, B, C, D, and E were chosen according to the EP. A reference solution was prepared as follows:

Step 1 – Preparation of individual stock solutions of each impurity at 1 mg/mL: 10 mg of each impurity was dissolved in 2 mL acetonitrile and was then diluted to 10 mL with mobile phase A.

Step 2 – Preparation of solution of all impurities at 0.06 mg/mL: 600 µL of each stock solution from step 1 was combined and the mixture was diluted to 10 mL with mobile phase A.

Step 3 – Preparation of reference solution: 20 mg of a certified reference standard (CRS) of ibuprofen was dissolved in 2 mL acetonitrile. Then 1 mL of the impurity solution from step 2 was added and the mixture was diluted to 10 mL with mobile phase A. The final concentrations were 2 mg/mL ibuprofen and 0.006 mg/mL (6 µg/mL) of each of the impurities.

Various dilutions of the reference solution were made to establish calibration curves for ibuprofen and the impurities.

Chromatographic conditions

- Column: Agilent ZORBAX SB-C18, 150 mm x 4.6 mm, 5 µm particle size
- Injection volume: 20 µL, as described in the EP regulations
- Column temperature: 30 °C, as described in the EP regulations
- Detector: 14 µL cell, peak width: 0.1 min (5 Hz), signal: 214 nm

Solvents, gradient, and pump settings

Solvent A was prepared by mixing 340 volumes acetonitrile, 0.5 volumes phosphoric acid, and 600 volumes water; then allowing to equilibrate and diluting to 1000 volumes with water. The procedure is described in the EP regulations. Solvent B was acetonitrile.

The gradient described in the EP regulations was as follows: 0-25 min 100 %A, 25-55 min 85 %B, 55-70 min 85 %B, 70-75 min 100 %A. Use of the EP gradient would lead to a runtime of 85 minutes. To achieve high resolution at short retention times with the column required by EP, the gradient time was reduced accordingly until all peaks were eluted (in 42.5 minutes including backflushing) with the same gradient slope, so that the same elution could be achieved as with the long gradient.

The final gradient and pump parameters were as follows:

- Gradient: 0-25 min 100 %A, 25-35 min 28.3 %B, 35.1-42.5 min 100 %A
- Stop time: 42.5 min
- Post time: 10 min (usually not necessary with the chosen conditions)
- Flow rate: 2 mL/min

System suitability and performance test

In accordance with Q3A(R) Impurities in New Drug Substances[3], the following parameters must be tested and the limit settings below must be fulfilled:

- Precision of areas must be < 2 % RSD.
- Precision of retention times must be < 0.5 % RSD.
- Resolution must be > 1.5 for all peaks.
- Signal-to-noise ratio must be > 50 for all peaks.
- Calibration for impurities and main peak must be linear without dilution.

With these requirements for testing, the samples shown in table 1 were prepared and analyzed.

An Agilent publication was used as a reference for this work.[4]

Results and discussion

The figure on the cover page shows an example chromatogram of ibuprofen and its impurities. The results of the control sample are shown in table 2. At the lowest calibration concentration, all criteria were fulfilled. The required sensitivity was obtained for all peaks and resolution > 1.5 was achieved for all compounds in the mixture.

Table 3 shows the precision of the areas and retention times for the main compound and the impurities in the suitability sample. The reliability and precision of the Agilent 1120 Compact LC system was proven. For all components, the criteria for precision of retention times and areas were fulfilled, showing that the system can be used for QC methods.

The results of all calibration runs are summarized in table 4. Linearity is shown from the lowest calibration concentration (10 % of the concentration of the reference solution) to the highest concentration (150 % of the concentration of the reference solution). No dilution of the main compound was necessary and no enrichment was required to detect the impurities.

The average calculated limit of detection (with S/N of 10) for all compounds was 0.10 µg/mL by using this method.
The disregard limit according to EP for each impurity is 0.05 times the area of ibuprofen in a reference solution that has a concentration of 20 µg/mL for ibuprofen and 1 µg/mL for each of the impurities. The results of the control sample showed a signal-to-noise ratio of > 50 at 0.6 µg/mL, so the requirements for limit of detection were fulfilled.
The excellent results shown here reflect a system that was designed for robust operation. The high performance of the new pump is strongly demonstrated by the good results for the precision of the retention times. Also, the high S/N ratios (> 50 for each component down to the disregard limit) are a result of the low pump pulsation. The high precision of the injector is shown with the good correlation results (r>0.999) during calibration, which is also proof of very low carryover.

Sample	Purpose	Number of injections
Blank solution	Verify baseline stability and identify artifacts	2
Calibration mixture 1-6	Verify stability of response and correctness of calibration, linearity	6 of each
Control sample	Verify sensitivity and resolution for lowest calibration sample	6
Suitability sample	Verify precision of areas and retention times for reference solution	6

Table 1
Setup for testing system suitability and performance.

	Retention time (min)	Amount	Resolution	S/N
Impurity D	5.573	0.6 µg/mL	3.89	56.9
Impurity C	10.783	0.6 µg/mL	16.29	223.7
Impurity A	27.516	0.6 µg/mL	27.82	128.8
Ibuprofen	28.874	0.2 mg/mL	1.98	5300
Impurity B	30.231	0.6 µg/mL	2.61	195.2
Impurity E	32.625	0.6 µg/mL	5.49	407.6

Table 2
Results for control sample: resolution and signal-to-noise (S/N) ratio.

Compound	Retention time (min)	Amount (µg/mL)	RSD RT n=6	RSD area n=6
Impurity D	5.573	6	0.225	0.089
Impurity C	10.783	6	0.267	0.535
Impurity A	27.516	6	0.222	0.159
Ibuprofen	28.874	2,000	0.448	0.061
Impurity B	30.231	6	0.142	0.236
Impurity E	32.625	6	0.078	0.088

Table 3
Suitability sample: precision of retention times and areas. (RSD = relative standard deviation; RT = retention time).

	m	b	Residual standard deviation	r
Impurity D	14872.3	11189.7	2778.9	1
Impurity C	19818.7	3086.1	19463.5	0.9999
Impurity A	24614.7	-40068	24769	0.99989
Ibuprofen	6417010	689238	895680.5	1
Impurity B	19969.4	-13173	7292	0.99999
Impurity E	35314.4	-19975.5	5740.8	1

Table 4
Calibration (Setting "Ignore Origin", y=mx+b, 0.6 to 9 µg/mL for impurities and 0.2 to 3 mg/L for ibuprofen).

Conclusion

The Agilent 1120 Compact LC is designed for users in medium- to small-size companies who need the highest reliability and ease-of-use, as well as the lowest cost-of-ownership for standard LC methodology in a QA/QC environment.

In order to prove precise results from a system that was optimized for every-day productivity, and to fulfill regulatory compliance, the experiments in this Application Note included determination of precision of areas and retention times, as well as chromatographic parameters like resolution, linearity, and signal-to-noise ratios. The results show that the instrument is within the limits of the regulatory requirements for a quality control environment.

The results show explicitly the applicability of the 1120 LC system for pharmaceutical testing in QA/QC departments. In addition to the instrument capabilities, the new version of the Agilent EZChrom Elite Compact software allows the full control of the Agilent 1120 Compact LC with a wide range of features for data analysis and reporting of the results.

References

1.
European Pharmacopoeia 4.2*, Council of Europe, 2737-9,* **2002.**

2.
"Agilent 1120 Compact Liquid Chromatograph: Overview, Specifications, Ordering," *Agilent Technologies publication number 5989-7454ENA,* **2008.**

3.
Q3A Impurities in New Drug Substances, Revision 2, http://www.fda.gov/cder/guidance/7838fnl.pdf, **2008**.

4.
Huesgen, A.G., "Impurity Profiling with the Agilent 1200 Series LC System. Part 5: QA/QC Application Example Using a Fast LC Method for Higher Sample Throughput," *Agilent Technologies publication number 5989-5621EN*, **2006.**

USP purity analysis of pravastatin sodium using the Agilent 1120 Compact LC

Application Note

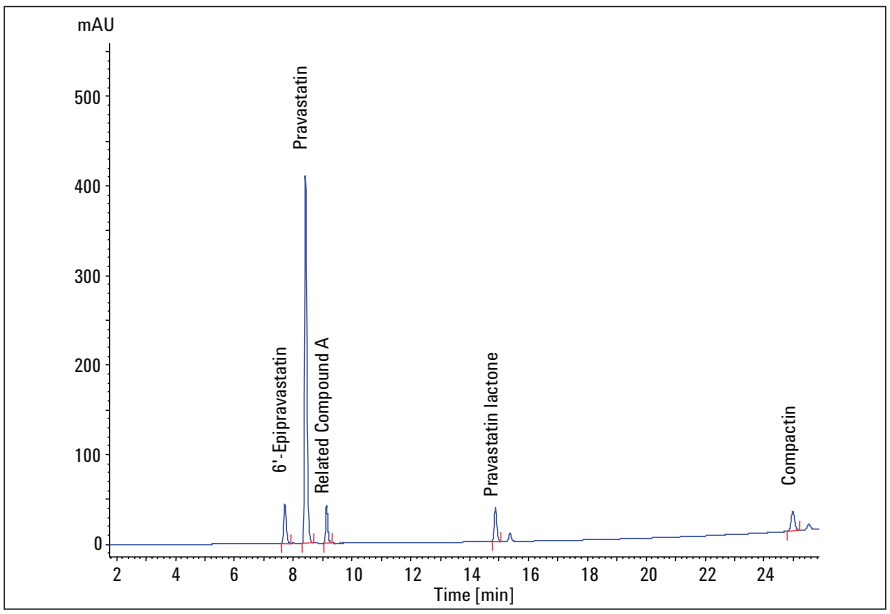
Manufacturing QA/QC

Authors

Syed S. Lateef, Siji Joseph

Agilent Technologies

Bangalore, India



Abstract

Pravastatin sodium helps to reduce cholesterol biosynthesis, thereby preventing cardiovascular disease. The chromatographic method for purity that was established by the United States Pharmacopeia (USP) for pravastatin sodium was performed on the Agilent 1120 Compact LC, and system suitability parameters were verified. The system suitability mixture containing pravastatin 1,1,3,3-tetramethylbutylamine and the impurity known as Related Compound A displayed a resolution of 6.3, which is well above the acceptance limit of not less than 2.0. The detector in the Agilent 1120 Compact LC produced a linear response of Related Compound A down to a concentration of 0.09 µg/mL.

Introduction

The USP chromatographic method for purity of pravastatin sodium[1] was performed on the Agilent 1120 Compact LC, and system suitability parameters were tested. The system suitability test sample consisted of USP pravastatin 1,1,3,3-tetramethylbutylamine and USP Related Compound A, which is a sodium salt for the impurity 3α-hydrox-yisocompactin. Pravastatin sodium and Related Compound A are positional isomers (figure 1).

Pravastatin sodium has six impurities that are reported by USP:

1. 3″-Hydroxypravastatin
2. 6 -Epipravastatin
3. 3α-Hydroxyisocompactin (also called Related Compound A)
4. Pentanoyl impurity
5. Pravastatin lactone
6. Compactin

Only impurities 2, 3, 5, and 6 were used to demonstrate the impurity method on the Agilent 1120 Compact LC.

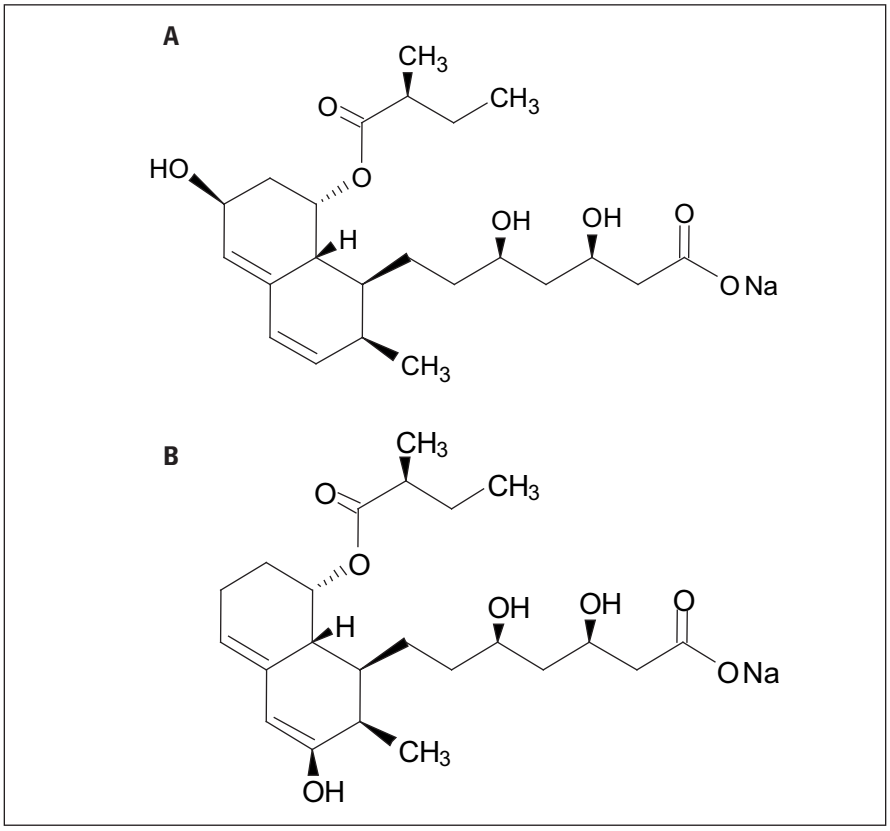


Figure 1
A) Pravastatin sodium. B) USP Related Compound A.

Experimental

Sample preparation

- **Diluent: A 1:1** mixture of methanol and water was prepared. The methanol was HPLC grade (J.T. Baker). Milli-Q water (Millipore) was used for the experiment.
- **System suitability sample:** Pravastatin 1,1,3,3-tetramethylbutylamine and Related Compound A were dissolved in diluent to obtain a concentration of 0.6 mg of pravastatin 1,1,3,3-tetramethylbutylamine and 0.001 mg of Related Compound A per mL. The system suitability sample was used to determine the value of the relative retention time (RRT) of Related Compound A versus pravastatin. Both pravastatin 1,1,3,3-tetramethylbutylamine and Related Compound A were obtained from the USP.
- **Standard sample:** Pravastatin 1,1,3,3-tetramethylbutylamine was dissolved in diluent to a concentration of 1.25 µg/mL. The standard sample was used to determine the relative standard deviation (RSD) of the peak areas.
- **RRT test sample:** Pravastatin sodium and four of its impurities – 6 -epipravastatin, Related Compound A, pravastatin lactone, and compactin – were dissolved in diluent to a concentration of 65 µg/ml for pravastatin and 7.5 µg/mL for the impurities. The RRT test sample was prepared to determine the RRT of each impurity, for comparison with values reported in the USP. Pravastatin lactone and compactin were obtained from VARDA Biotech. Related Compound A was obtained from the USP and 6′-epipravastatin was obtained from the European Pharmacopeia (EP).
- **Detector linearity test samples:** Seven concentration levels of Related Compound A from 0.09 µg/mL to 1.20 µg/mL were prepared as spiked amounts in the pravastatin sodium sample, which had a constant concentration of 0.5 mg/mL. These samples were used to test the detector response for linearity at low concentrations of the impurity.

Equipment

The Agilent 1120 Compact LC system included:

- A binary pump with integrated degasser
- An autosampler with vial tray
- A variable wavelength detector (VWD)

The instrument was controlled by the Agilent EZChrom Elite Compact software.

Results and Discussion

System suitability results

According to the USP monograph on pravastatin sodium, the system suitability sample containing pravastatin 1,1,3,3-tetramethylbutylamine and Related Compound A should show a resolution of not less than (NLT) 2.0 and Related Compound A should show an RRT of about 1.1. (USP suggests that there are no acceptance criteria applied to RRT, as RRT is an aid in peak identification only.)

Figure 2 shows six replicate injections of the system suitability sample. The results show that Related Compound A has an RRT of 1.1 for all runs and a resolution of 6.3, which is better than the USP limit of NLT 2.0.

Standard deviations of pravastatin peak areas

The standard sample containing 1.25 µg/mL of pravastatin 1,1,3,3-tetramethylbutylamine was injected six times to test the relative standard deviation of the peak areas of pravastatin. According to the USP method, six replicate injections of the standard sample should have a relative standard deviation (RSD) of the peak areas of not more than (NMT) 10.0 %. A value of 0.3 % RSD was obtained, suggesting the excellent precision of the injector in the Agilent 1120 Compact LC. Figure 3 shows the six injections from the standard sample.

Retention times of the impurities relative to the pravastatin retention time

Figure 4 shows the chromatogram of the RRT test sample analyzed according to the USP test for chromatographic purity for pravastatin. The test was used to calculate RRTs and compare them with the RRTs provided by the USP. Table 2 shows the comparison and excellent match between the RRTs obtained experimentally and those reported by the USP.

Parameters	Detail	
Wavelength for VWD	238 nm	
Column	Agilent ZORBAX SB-C18, 4.6 x 75 mm, 3.5 µm	
Diluent	50:50 methanol:water	
Injection volume	10 µL	
Needle wash	Flush port, 3 sec using diluents	
Sample temperature	Ambient	
Column temperature	25 °C	
Mobile phase	Buffer pH 7.0: 0.08 M phosphoric acid solution adjusted with triethylamine to pH 7.0	
Buffer A:	Water:buffer pH 7.0:acetonitrile (52:30:18)	
Buffer B:	Water:buffer pH 7.0:acetonitrile (10:30:60)	
Gradient	Time	% Buffer B
	0	0
	3.0	0
	26.5	100
	26.6	0
	30	0
Post-time	3 min	
Flow	1 mL/min	

Table 1
Instrument parameters.

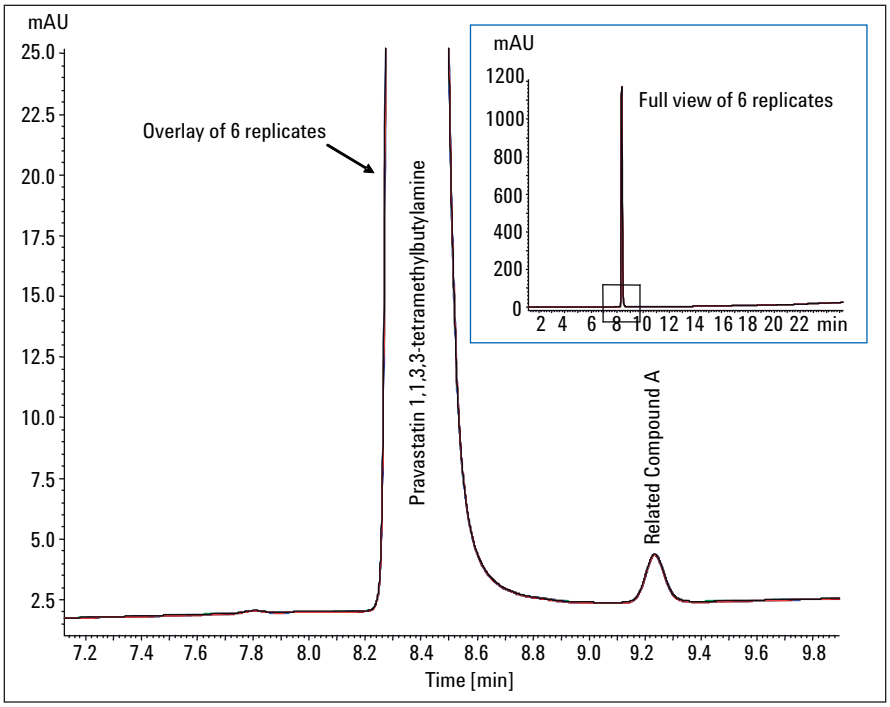


Figure 2
Overlay of six replicate injections of the system suitability sample. The insert shows the full view of the replicate injections.

Name	USP-reported relative retention time	Experimentally obtained relative retention time
3″-Hydroxypravastatin	0.33	-*
6′-Epipravastatin	0.92	0.91
3α-Hydroxyisocompactin	1.1	1.1
Pentanoyl impurity	1.2	-*
Pravastatin lactone	1.8	1.8
Compactin	3.1	3.1

Table 2
Experimentally determined RRTs of four out of the six impurities reported in the USP method.

Results of detector linearity tests
The USP method specifies an upper limit concentration of 0.2 % for Related Compound A, which is 1 µg/mL based on a concentration of 0.5 mg/mL for the pravastatin sodium solution. To test the detector's linear response at low levels, 0.09 µg/mL to 1.20 µg/mL of Related Compound A was spiked into 0.5 mg/mL of pravastatin sodium. Table 3 shows signal-to-noise (S/N) ratios obtained at each of the spiked levels. The spiked level with a concentration of 0.17 µg/mL, which corresponds to 0.034 %, is the limit of quantification (LOQ). Figure 5 shows the overlaid chromatograms at each of the injection levels. A uniform rise of the injection amount was seen, demonstrating excellent detector response for the Agilent 1120 Compact LC. The linearity plot of the injection levels is shown in Figure 6, where the correlation coefficient (R^2) for the linearity is 0.998.

Conclusion

The method from the USP monograph on chromatographic purity for pravastatin sodium was performed on the Agilent 1120 Compact LC system. The system suitability results showed excellent resolution of 6.3. Injector precision at low concentration levels of pravastatin was demonstrated from replicate injections of the standard sample; an excellent value of 0.3 % was obtained for the RSD of the areas. A linearity value (R^2) of 0.998 was observed for the Related Compound A impurity at levels lower than its limit concentration, demonstrating outstanding detector response.
USP methods are validated methods and do not require revalidation, but do require simple verification of the method. The results of this study clearly show that the USP method was verified on the Agilent 1120 Compact LC.

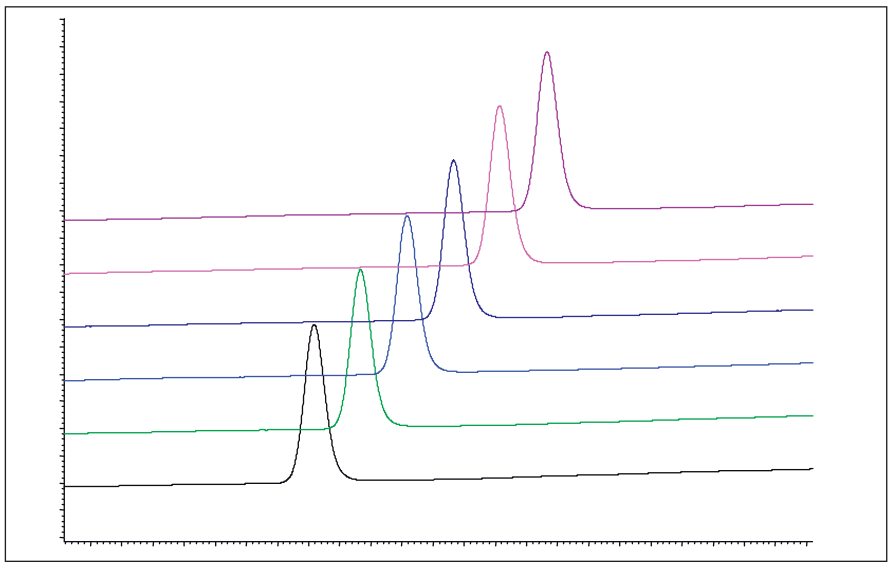


Figure 3
Six replicate injections of the standard sample (x and y axes offset).

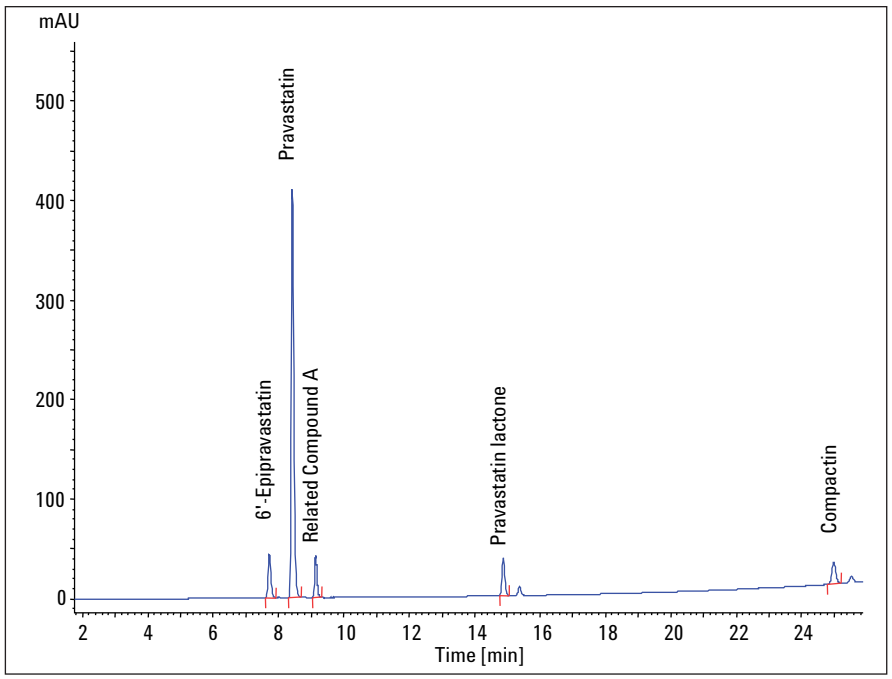


Figure 4
Chromatogram of the RRT test sample. The peaks next to pravastatin lactone and compactin originate from the impurities.

¡References

1.
U.S. Pharmacopeia 31-NF 26, second supplement, 2008.
http://www.uspnf.com/uspnf/pub/index

Development, validation, and comparison of an HPLC method to analyze paracetamol and related impurities according to the European Pharmacopoeia (EP) and USP using the Agilent 1120 Compact LC and the Agilent 1200 Series LC system

Application Note

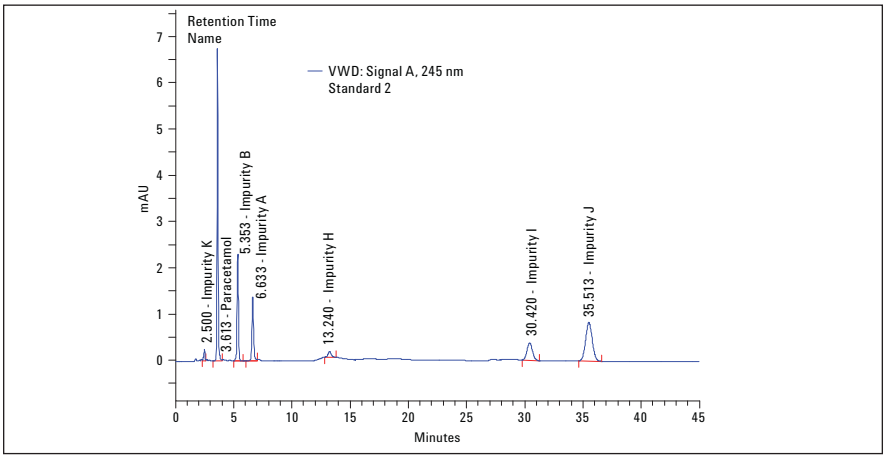
Pharmaceuticals

Author

Detlef Wilhelm

ANATOX GmbH & Co. KG

Fuerstenwalde, Germany



Abstract

This Application Note compares the results of the development of an accurate and reproducible method to analyze paracetamol and related impurities according to European Pharmacopoeia (EP) and USP regulations[1], using an Agilent 1120 Compact LC and a 1200 Series LC system. The experiments described in this Application Note include the determination of the precision of areas and retention times, as well as chromatographic parameters such as resolution and signal-to-noise ratios. The well-suited Agilent ZORBAX StableBond RP8 column shows good selectivity for this application.

The results of these experiments prove that the Agilent 1120 Compact LC is a reliable instrument for routine testing that is able to fulfill the requirements of the European and U.S. regulations and can produce results comparable to those of a standard Agilent 1200 Series LC system.

Introduction

The analytical instrumentation for routine analysis of samples with standardized LC methods, especially for quality control testing, have several requirements. Foremost are high reliability, ease of use, and an optimal cost of ownership.

This Application Note shows how the Agilent 1120 Compact LC, in comparison with a standard Agilent 1200 Series LC system, works as a highly robust and reliable instrument for standard LC methodology and can be used effectively in a routine environment to efficiently measure pharmaceutical compounds, such as paracetamol and related impurities.

According to the EP regulations, paracetamol impurities A, B, F, H, I, J, and K were analyzed on both LC systems, and system suitability and performance tests were executed.

Experimental

Instrumentation

An Agilent 1120 Compact LC system and a standard Agilent 1200 Series LC system with the following configurations were used:

Configuration of the 1120 Compact LC	Configuration of the 1200 Series system
Gradient pump and vacuum degasser	Quaternary pump and vacuum degasser
Auto sampler	Standard autosampler
Column oven	Column compartment
Variable wavelength detector	Diode array detector
Software: EZChrom Elite Compact 3.3	Software: ChemStation B.04.01

Preparation of samples

The reference solution was prepared as follows, in accordance with EP regulations. 5 mg of paracetamol and 5 mg of each impurity were dissolved in methanol and diluted to 20 mL with the same solvent. 1 mL of the solution was diluted to 250 mL with mobile phase. The substances to be checked were paracetamol and impurities K, A, B, H, I, J, and F.

Chromatographic conditions

Column	Agilent ZORBAX StableBond-C8, 4.6 x 250 mm, 5 µm
Mobile phase	Mix together 375 mL of a 17.9 g/L solution of disodium hydrogen phosphate, 375 mL of a 7.8 g/L solution of sodium dihydrogen phosphate, and 250 mL of methanol containing 6 mL of a 400 g/L solution of tetrabutylammonium hydroxide in methanol
Pump settings	No gradient (in accordance with EP regulations)
Stop time	45 min
Flow rate	1.5 mL/min, isocratic
Injection volume	20 µL
Column compartment temp.	35 °C
Detector	
1120 LC system	14 µL
1200 Series system	13 µL
Peak width	0.1 min (5 Hz)
Signal	245 nm

System suitability and performance test

The EP regulations for paracetamol require system suitability testing with a reference solution, as described in Preparation of samples, above. The testing included the following limits:

Resolution	4.0 minimum between peaks to impurity K and to paracetamol
Signal-to-noise ratio	50 minimum for the peak due to impurity J
Relative retentions (paracetamol)	Impurity K = 0.8
	Impurity F = 3
	Impurity J = 7

No special regulations for paracetamol come from the USP. However, according to USPC Official 8/1/08, General Chapter <621> (Chromatography, System Suitability, p. 28) if there are no special requirements in the monographs, the data of five replicate injections should have a relative standard deviation of less than 2% for each calculated parameter.

From these above-mentioned requirements and to check and compare the chromatographic performance of both LC systems, the following parameters were tested and the limit settings below were fulfilled:

• Precision of areas must be < 2% RSD

• Precision of retention times must be < 0.5% RSD

• Resolution must be > 4 for impurity K and Paracetamol

• Signal-to-noise ratio must be > 50 for impurity J

With these limits and settings for testing, the samples in Table 1 were prepared and analyzed.

Sample	Purpose	Number of injections
Blank solution	Verify baseline stability and identify artifacts	2
Control sample	Verify sensitivity and resolution for reference solution	6
Suitability sample	Verify precision of areas and retention times for reference solution	10

Table 1
Setup for testing.

Results and discussion

Figure 1 shows a chromatogram achieved with the 1200 Series system and ChemStation, whereas Figure 2 shows the chromatogram yielded with the 1120 Compact LC and EZChrom Elite Compact. The data for both chromatograms, shown in Tables 2 and 3, are very similar.

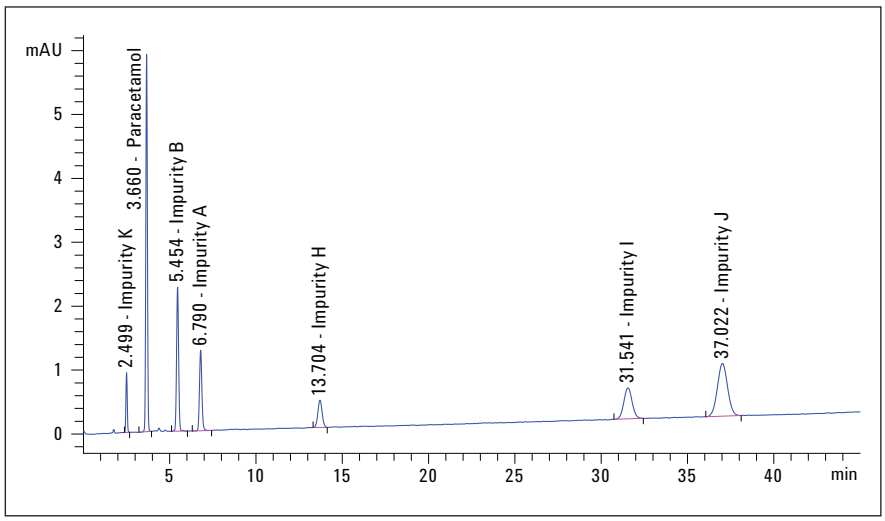


Figure 1
Example chromatogram of paracetamol and impurities with the Agilent 1200 Series system.

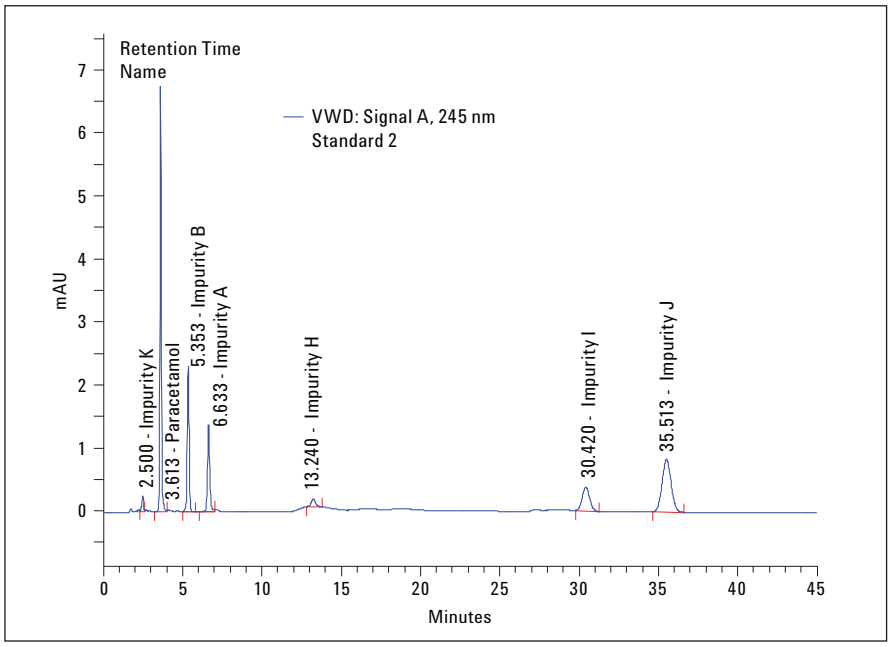


Figure 2
Example chromatogram of paracetamol and impurities with the Agilent 1120 Compact LC.

As shown in Figures 1, 2, and 3, the resolution and relative retention meet the EP requirement for both paracetamol and impurity F.

The results of the control sample, shown in Table 2, fulfill all criteria. The sensitivity was given for all peaks and resolution was achieved for all relevant compounds of the mixture. Not only for impurity K and paracetamol, but for all other relevant peaks the resolution was greater than 4, showing very good selectivity for the ZORBAX StableBond RP8 material and the good performance of the system. The data for peak symmetry (not shown) for all peaks ranged from 0.88–1.02.

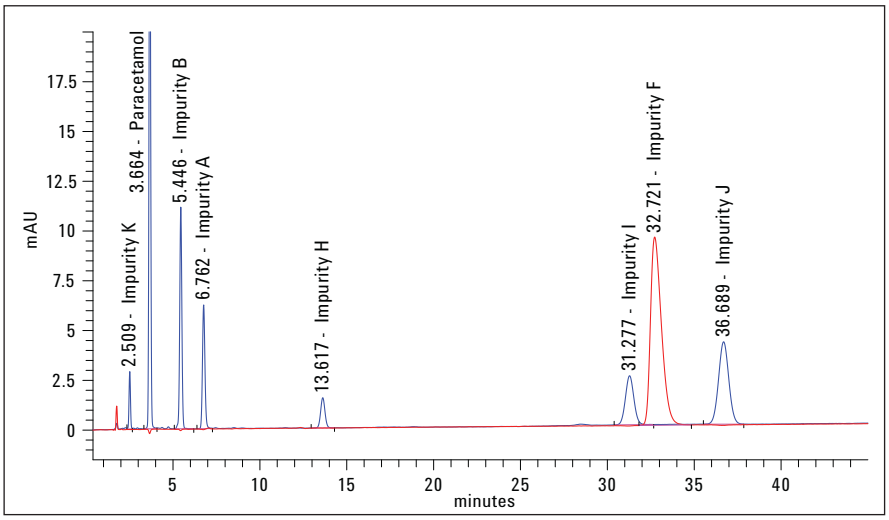


Figure 3
Chromatogram with all relevant impurities.

Compound	Retention time (minutes) Agilent 1200 Series LC system	Retention time (minutes) Agilent 1120 Compact LC	Resolution Agilent 1200 Series LC system	Resolution Agilent 1120 Compact LC
Impurity K	2.498	2.507	–	–
Paracetamol	3.659	3.620	7.19	7.03
Impurity B	5.454	5.353	9.22	9.04
Impurity A	6.792	6.633	5.75	5.51
Impurity H	13.716	13.240	19.52	18.35
Impurity I	31.562	30.387	26.25	24.89
Impurity J	37.066	35.480	5.47	5.03

Table 2
Results for control sample: retention times and resolution.

The criterion for the signal-to-noise ratio for impurity J was fulfilled for both systems. With the Agilent 1200 Series system the average value was found to be 61.4 and with the Agilent 1120 Compact LC system, it was 63.9 for the reference solution.

Table 3 shows the areas and retention time precision results of the main compound and the impurities of the suitability sample. The reliability and precision of the Agilent 1200 Series and the Agilent 1120 Compact LC system were proven. For all components the criteria for precision of retention times and areas were fulfilled, so that both systems can be used for QC methods.

Comparing the results for the suitability sample, it was found that the precision of retention times was nearly the same. Only few deviations were observed. The same was seen with the precision of areas. Both systems provide for the same injector and detector performance, independent of the hardware.

	Agilent 1200 Series LC system			Agilent 1120 Compact LC		
Compound	Retention time (min)	RSD RT n = 25	RSD Area n = 10	Retention time (min)	RSD RT n = 25	RSD Area n = 10
Impurity K	2.498	0.127	0.656	2.507	0.177	0.690
Paracetamol	3.659	0.048	0.545	3.620	0.111	0.342
Impurity B	5.454	0.061	0.263	5.353	0.135	0.352
Impurity A	6.792	0.154	0.215	6.633	0.206	0.348
Impurity H	13.716	0.22	0.831	13.240	0.311	0.636
Impurity I	31.562	0.278	0.599	30.387	0.371	0.681
Impurity J	37.066	0.324	0.404	35.480	0.386	0.258

Table 3
Suitability sample: precision of retention times and areas.

Conclusion

The Agilent 1120 Compact LC is designed for users who need the highest reliability, ease of use, and lowest cost of ownership for standard LC methodology in a QA/QC environment in medium to small companies. The comparison with the standard 1200 Series LC system shows very similar results for these applications.

To prove precise results from a system optimized for everyday productivity and to fulfill regulatory compliance, the experiments in this Application Note included determination of precision of areas and retention times, as well as chromatographic parameters like resolution and signal-to-noise ratios.

As shown in Table 2, the resolution of all peaks was found to be greater than 4.0, with a signal-to-noise ratio of more than 60 (> 50 required) with both systems for the relevant compound. The calculated signal-to-noise ratios prove the sensitivity of the system and show that the instrument can be operated according to the requirements in a quality control environment.

The results of Table 3 show that all criteria for the precision of the determination (areas and retention times) are fulfilled. All criteria related to EP and USP requirements are fulfilled, and the determination of paracetamol and its impurities can also be done with the reliable Agilent 1120 Compact LC system.

All results explicitly show the applicability of the 1120 Compact LC system for drug testing in QA/QC departments due to reduced costs per system and improved simplicity of use. In addition to the instrument capabilities, the new version of the EZChrom Elite Compact software allows full control of the Agilent 1120 Compact LC, with a wide range of features for data analysis and reporting of the results.

The high performance of the new pump is strongly demonstrated by the good results for the precision of the retention times. Also the high S/N ratios (> 50 for the relevant components with the reference sample) are a result of the low pump pulsation. The high precision of the injector is shown with very similar results for area precision compared to the standard LC system.

The results for peak symmetry show the good selectivity and performance of Agilent column technology as well as the very good flow design of the LC systems, with no band broadening or peak distortion.

Reference

1. European Pharmacopoeia 5.0, 2184–5

Determination of degradation products of Metoprolol tablets with the Agilent 1120 Compact LC after method development with the Agilent 1200 Series Rapid Resolution LC system

Application Note

Pharmaceuticals

Authors

Detlef Wilhelm
ANATOX GmbH & Co. KG
Fuerstenwalde
Germany

Abstract

Conventional LC methods used in routine analyses to characterize or monitor chemicals are often standardized for quality control of pharmaceutical products. This Application Note describes the analysis of several degradation products of metoprolol with the Agilent 1120 Compact LC. The preceding method development was done with the Agilent 1200 Series Rapid Resolution LC (RRLC) system.

This Application Note starts with the final result of method development, to prove that no further development is necessary.[1] It shows that the Agilent 1120 Compact LC works as a reliable and highly robust instrument for standard LC methodology. The transfer from rapid resolution separation material to conventional columns by the Method Translator Software is shown. The results of system suitability and performance tests prove the reliability and quality of the Agilent 1120 Compact LC system for everyday quality control testing.

Introduction

The Agilent 1120 Compact LC was designed to meet the highest requirements for analytical instrumentation used in routine analysis. In the field of quality control testing, products are often characterized with standard LC methods. Therefore, instruments used for these purposes need optimal cost-of-ownership, high reliability, high flexibility, and ease of use.

In the last few years the method development of new tests with a high-end Agilent 1200 Series RRLC system became familiar. In the Application Note "Agilent 1200 Series LC Method Development Solution for the analysis of degradation products of metoprolol tablets, Agilent Technologies publication 5989-9339EN" the results of the Agilent method development solution are shown.

This Application Note starts with the final results of method development, to prove that no further enhancement is necessary. It then shows that the Agilent 1120 Compact LC works as a reliable and highly robust instrument for standard LC methodology. It also shows that the Agilent 1120 Compact LC can be used for determinations after back transfer from rapid resolution separation material to conventional columns by the Agilent Method Translator Software. The results of system suitability and performance tests in this Application Note prove the reliability and quality of Agilent 1120 Compact LC for everyday quality control testing.

Instrumentation

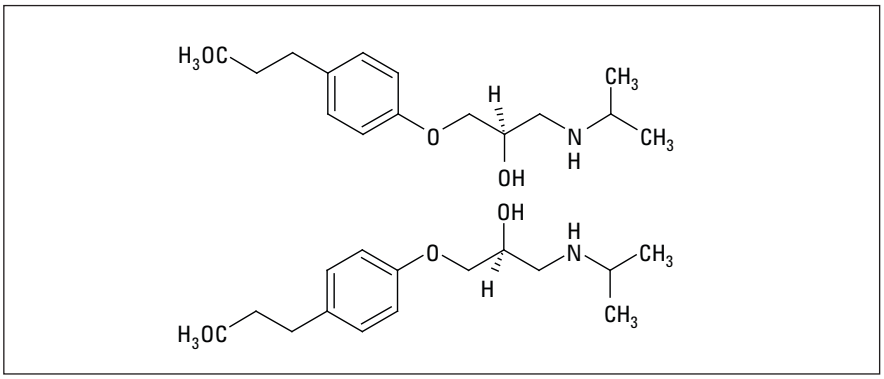
For method development an Agilent 1200 Series RRLC system and an Agilent 1120 Compact LC with the following configurations were used:

Configuration of the Agilent 1200 Series Rapid Resolution LC System	Configuration of the Agilent 1120 Compact LC
Binary pump (low delay configuration) and vacuum degasser	Gradient pump and vacuum degasser
Wellplate autosampler	Auto sampler
2 Column compartments	Column oven
Diode array detector	Variable wavelength detector
Software: ChemStation B.04.01	Software: EZ-Chrom Elite Compact 3.3

Preparation of samples

Reference samples

Samples were prepared in the same manner as those described in another Application Note.[1] Two 50-mg metroprolol tablets were powdered. One of the samples was dissolved in water. The other tablet was heated to 80 °C for 3 hours, and the residue also dissolved in water. Both solutions were two step filtered by syringe filters: first by an Agilent p/n 5064-8222, 2-µm filter followed by an Agilent p/n 5064-8221, 0.45-µm filter. Five microliters of the resulting liquids were injected.



Structure of Metoprolol (both isomers)

Chromatographic conditions		
Column		
For method development:	Agilent ZORBAX StableBond C18, 50 mm ˘ 2.1 mm, 1.8 µm	
For routine testing:	Agilent ZORBAX StableBond C18, 150 mm ˘ 4.6 mm, 5 µm	
Mobile Phase A:	Water + 0.2% TFA	
Mobile Phase B:	Acetonitrile + 0.16% TFA	
Gradient (linear):	Time (min)	% B
	0	5
	45	50
Pump settings		
Stop time:	45 min	
Post time:	5 min	
Flow rate:	2.0 mL/min	
Autosampler		
Injection volume:	15 µL	
Thermostatted column compartment		
Temperature:	30 °C	
Detector	14 µL cell, Peak width: >0.05 min, 1 s response time (10 Hz), Signal: 210 nm	

System suitability and performance test

The following limits were used for system suitability testing the reference samples (see Reference Sample Preparation):

Similar peak pattern according to separation with rapid resolution

Precision of areas must be < 2 % RSD.

Precision of retention times must be < 0.5 % RSD.

The following samples were prepared and analyzed, using the limits and settings recorded in Table 1.

Sample	Purpose	Number of injections
Blanc solution	Verify baseline stability and identify artifacts	2
Suitability sample	Verify precision of areas and retention times for reference solution	10

Table 1
Setup for testing.

Results and discussion

Conditions for the method transfer were chosen from the Agilent 1200 Series RRLC method development system (see Preparation of samples).

The first step was to select the same column selectivity and column efficiency. The efficiency for the 50 mm ˇ 2.1 mm, 1.8 µm rapid resolution high throughput (RRHT) column was estimated to nearly 12000 plates whereas for the 150 mm ˇ 4.6 mm, 5 µm conventional column, 10700 plates were calculated. Agilent provides materials with the same selectivity independent of the particle size. As a final result of method development, the Agilent ZORBAX StableBond C18 material was found to separate all compounds.

According to these data, the RRLC parameters were transferred into the Agilent Method Translator to transform them to parameters suitable for conventional HPLC systems by selecting the simple conversion option (see Figure 1).

The Agilent Method Translator software calculates the new LC parameters, which should be used as starting conditions. Some parameters such as the different delay volumes of the pumps and different types of gradient mixing (high pressure versus low pressure) could not be converted satisfactorily. Sometimes these parameters must be adapted by experimentally optimizing the gradient steps.

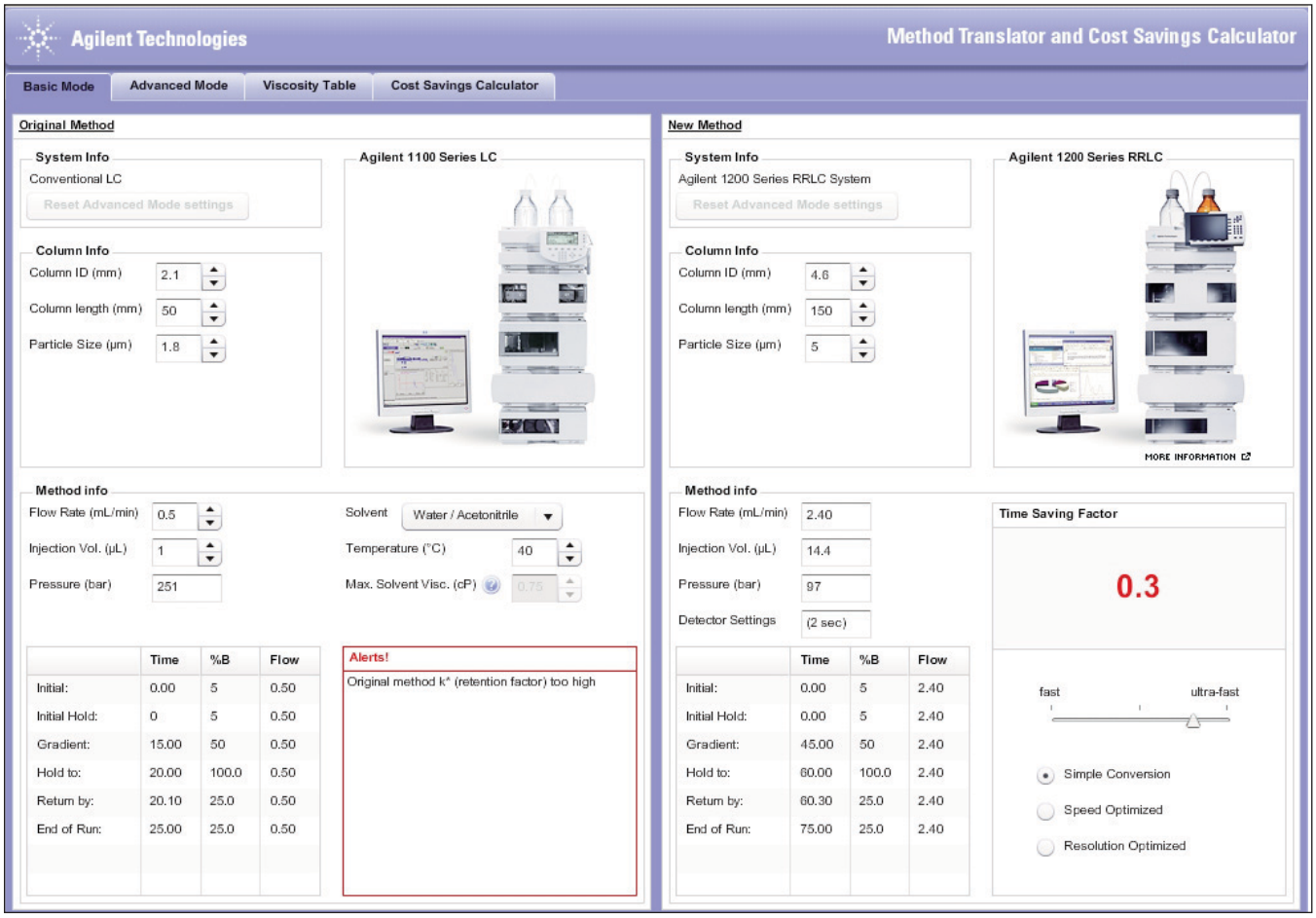


Figure 1
Conversion of LC parameters found by rapid resolution to parameters suitable for conventional HPLC with Method Translator software.

After transfering the parameters from the 50 mm ˇ 2.1mm, 1.8 µm RRHT column to the 150 mm ˇ 4.6 mm, 5 µm column, the analysis of pure metoprolol tablets with the Agilent 1120 Compact LC system and EZ-Chrom Elite Compact Software obtained chromatograms similar to those in Figure 2. As a result of the Method Translator, the flow was set to 2 mL/min, which prolonged the analysis time without any influence on the resolution.

The chromatogram in Figure 3 shows the appropriate separation of the degradation products from the Metoprolol peak. With the variable wavelength detector, several peaks at 210 nm are detected. The pattern looks very similar to the pattern obtained after separation with the Agilent 1200 Series RRLC system (see “Preparation of samples”).

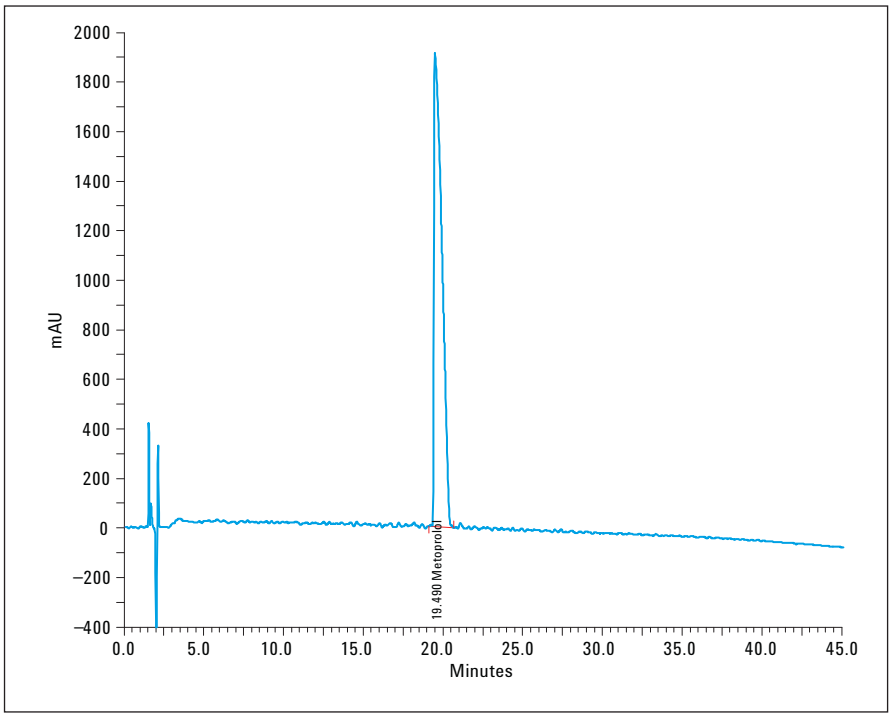


Figure 2
Analysis of metoprolol tablet after powdering, dissolving in water and filtering.

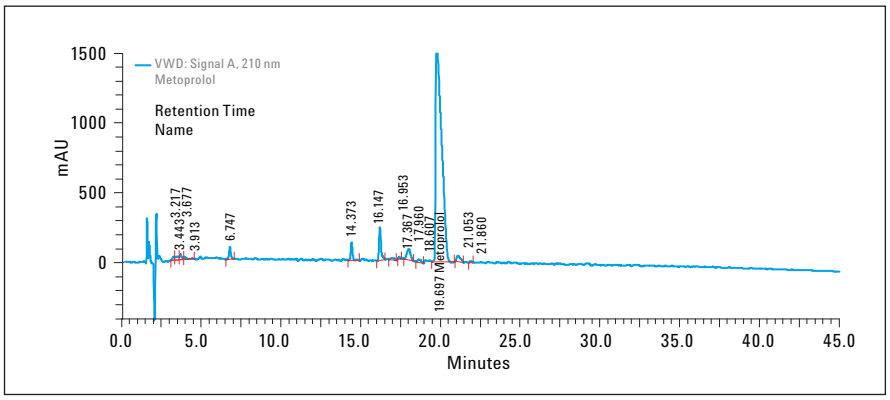


Figure 3
Analysis of a "stressed" metoprolol tablet after powdering, dissolving in water and filtering.

Compared to the results obtained with the Agilent 1200 Series RRLC system (see Table 2) the chromatogram in Figure 3 shows that the elution order has not changed. It shows that the selectivity does not change with the particle size. Detailed data for some selected peaks (see Figure 4) are listed in Table 3. As a main result it can be emphasized that it is possible to transfer LC parameters from rapid resolution by the Method Translator to conventional LC systems and columns with 5-µm particles.

The adaptation of method development results with rapid resolution to the Agilent 1120 Compact LC was successful. The appropriate separation with the Agilent ZORBAX StableBond C18 material is proven by the data shown in Table 3. High resolution for every peak > 2 shows that this column material is highly suitable for the determination of the degradation peaks of Metoprolol.

To demonstrate the reliability and precision of the Agilent 1120 Compact LC the suitability sample was analyzed 10 times. The data presented in Table 4 show the areas and retention time precision results of all compounds.

To use the system for QC methods the criteria (see System suitability and performance test) must be fulfilled for all compounds due to strong limits . The data for precision of retention times and areas show that all data are within the limits.

The high precision of retention times for all components is also a result of performance and reliability of the pumping system. The high reproducibility of the autosampler is best shown by the data for area precision. These data allocate the Agilent 1120 Compact LC to be used for QC testing.

Parameter	Agilent 1200 SL System	Agilent 1120 Compact LC System
Column-dimensions	50 mm x 2.1 mm	150 mm x 4.6 mm
Particle-size	1.8 µm	5 µm
Flow	0.50 mL/min	2.0 mL/min
Temperature	30 °C	30 °C
Gradient time	0-15 min	0–45 min
Composition	5% to 50%	5% to 50%
Delay volume	Low delay (120 µL)	Normal delay (approx. 1 mL)

Table 2
Detailed parameters for instrument setup.

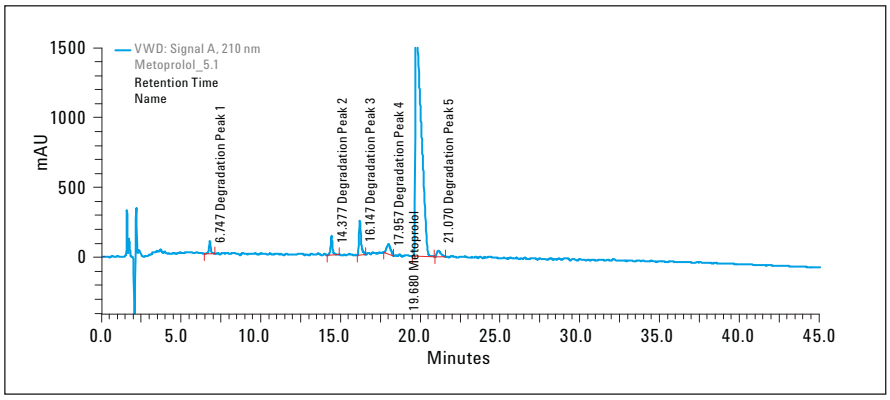


Figure 4
Selection of some representative peaks of a "stressed" metoprolol tablet for evaluating the stability of the separation.

Compound	Retention-time (min)	Resolution
Degradation peak 1	6.747	–
Degradation peak 2	14.377	37.84
Degradation peak 3	16.147	8.02
Degradation peak 4	17.957	5.23
Metoprolol	19.680	2.78
Degradation peak 5	21.070	2.25

Table 3
Results for retention times and resolution.

Compound	Retention-time (min)	RSD RT n=10	RSD Area n=10
Degradation peak 1	6.747	0	0.633
Degradation peak 2	14.377	0.023	0.747
Degradation peak 3	16.147	0.016	0.702
Degradation peak 4	17.957	0.048	0.601
Metoprolol	19.680	0.063	0.664
Degradation peak 5	21.070	0.048	0.895

Table 4
Suitability sample: Precision of retention times and areas .

Conclusion

The Agilent 1120 Compact LC was designed for conventional chromatography. Users from medium to small size companies, who have high requirements of reliability, ease-of-use and lowest cost-of-ownership for standard LC methodology in a QA/QC environment should be supported with this compact nonmodular LC system.

This Application Note shows the easy transfer of parameters determined by method development with Rapid Resolution (see Table 2). This approach is reliable and provides precision of areas and retention times, and high chromatographic resolution. The results show a system optimized for everyday productivity, which meets the highest requirements for routine analysis.

With the results shown in Table 3 the resolution of all main peaks was found to be greater than 2. The results in Table 4 show that all criteria for the precision of determination, such as area and retention times are fulfilled. The results allow the use of the Agilent 1120 Compact LC in QA/QC laboratories to determine degradation products of Metoprolol tablets.

All results show the applicability of the Agilent 1120 Compact LC for quality control testing with reduced costs per system and improved simplicity of use. In addition to the instrument capabilities, the new version of the EZChrom Elite compact software allows the full control of the Agilent 1120 Compact LC with a wide range of features for data analysis and reporting of results.

The high resolution results show that selectivity and performance of the Agilent ZORBAX StableBond material is independent of the particle size. The data also show that the design of the LC systems eliminates band broadening and peak distortion, and enhances the method transfer. In summary, an Agilent 1200 Series RRLC system provides fast method development and back transfer of results to conventional HPLC. The Agilent 1120 Compact LC is qualified for such an approach and meets the highest requirements for ordinary productivity.

References

1.
Agilent 1200 Series LC Method Development Solution for the analysis of degradation products of Metoprolol tablets, Agilent Technologies publication 5989-9339EN, **2009**

Specifications of the Agilent 1220 Infinity LC System

Pump	
Hydraulic system	Dual plunger in series pump with proprietary servo-controlled variable stroke drive, floating plungers and passive inlet valve
Settable flow range	0.001 – 10 mL/min, in 0.001 mL/ min increments
Flow range	0.2 – 10 mL/min
Flow precision	<0.07 % RSD, or < 0.02 min SD whatever is greater, based on retention time at constant room temperature
Flow accuracy	± 1 % or 10 µL/min whatever is greater
Pressure	Operating range 0 – 60 MPa (0 – 600 bar, 0 – 8700 psi) up to 5 mL/min Operating range 0 – 20 MPa (0 – 200 bar, 0 – 2950 psi) up to 10 mL/min (all versions)
Pressure pulsation	< 2 % amplitude (typically < 1 %), at 1 mL/min isopropanol, at all pressures > 1 MPa (10 bar)
Recommended pH range	1.0 – 12.5, solvents with pH < 2.3 should not contain acids which attack stainless steel
Gradient formation (gradient pump or optional; two solvents)	Low pressure dual mixing/gradient capability using proprietary high-speed proportioning valve Delay volume 800 – 1100 µL, dependent on back pressure
Composition range	0 – 95 % or 5 – 100 %, user selectable
Sampler	
Pressure	Operating range: 0 – 60 MPa (0 – 600 bar, 0 – 8700 psi)
Injection range	0.1 – 100 µL in 0.1 µL increments Up to 1500 µL with multiple draw (hardware modification required)
Replicate injections	1 – 99 from one vial
Precision	< 0.25 % RSD from 5 – 100 µL, < 1% RSD 1 – 5 µL variable volume
Minimum sample volume	1 µL from 5 µL sample in 100 µL microvial, or 1 µL from 10 µL sample in 300 µL microvial
Carryover	Typically < 0.1 %, < 0.05 % with external needle cleaning
Sample viscosity range	0.2 – 50 cp
Sample capacity	100 × 2-mL vials in 1 tray 40 × 2-mL vials in ½ tray 15 × 6-mL vials in ½ tray (Agilent vials only)
Injection cycle time	Typically 50 s depending on draw speed and injection volume

Column Oven	
Temperature range	5 °C above ambient to 80 °C
Temperature stability	± 0.15 °C
Temperature accuracy	± 0.8 °C with calibration ± 0.5 °C
Column capacity	One 25-cm column
Variable Wavelength Detector (G4286B, G4288B, G4290B)	
Light source	Deuterium lamp
Wavelength range	190 – 600 nm
Short term noise	± 0.35 × 10^{-5} AU, at 230 nm
Drift	3 × 10^{-4} AU/hr, at 254 nm
Linearity	> 2 AU (5 %) upper limit
Wavelength accuracy	± 1 nm; self-calibration with deuterium lines, verification with holmium oxide filter
Band width	6.5 nm typical
Diode Array Detector (G4294B only)	
Detector type	1024-element diode array
Light source	Deuterium and tungsten
Number of signals	8
Maximum sampling rate	80 Hz
Short-term noise	< ± 0.7 x 10^{-5} AU at 254/4 nm and at 750 nm, TC 2 s
Drift	< 0.9 x 10^{-3} AU/hr at 254 nm
Linearity	> 2.0 AU (5 %) at 265 nm
Wavelength range	190-950 nm
Wavelength accuracy	± 1 nm, self-calibration with deuterium lines verification with holmium oxide filter
Slit width	Programmable: 1, 2, 4, 8, 16 nm
Diode width	< 1 nm

www.agilent.com/chem/1220

Published in the USA, April 1, 2013
5991-1023EN

Agilent Technologies